• Volume 10A, Number 4, December 1961

BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

Section A
CHEMISTRY

Bull. Res. Counc. of Israel A. Chemistry

This issue is dedicated to
PROFESSOR MAX FRANKEL
on the occasion of his sixtieth birthday

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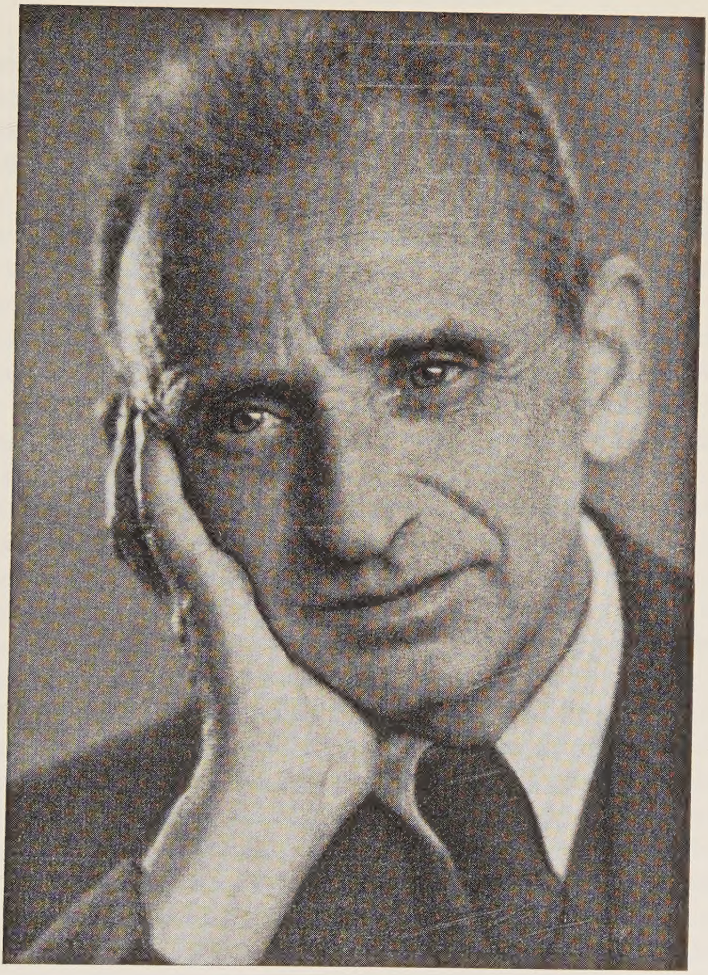

Professor Max Frankel

PROFESSOR MAX FRANKEL

This issue of the Bulletin is dedicated to Prof. Max Frankel on his 60th birthday, by his colleagues, pupils and friends.

Prof. Frankel is one of the teachers of the Hebrew University who laid the foundations for the development of science in Israel. He taught many students, moulded their characters and set standards for research. He holds an esteemed position in Israel's science, as a teacher and research scientist, as a pioneer scholar who devoted his life to the establishment of the Hebrew University and as a spiritual and scientific centre.

Frankel came to this country at the age of 25, after graduating from the University of Vienna with a Ph.D. for his research on the synthesis of dipyrimidines. In Austria he also taught and carried out research at the Institute of Chemistry and Chemical Technology, University of Vienna and at the Verein für Chemische und Metallurgische Produktion, Aussig.

He came to this country as a Zionist and a scholar to contribute his knowledge and experience to the development of science at the University. It was Weizmann's vision of establishing a cultural centre in Zion by combining science and technology that attracted the young scholar and stimulated him. Professor Fodor invited Frankel to join him on Mt. Scopus in the first laboratories of the University for biochemistry and colloidal and organic chemistry.

The organizational and administrative work encountered by every scientist trying to form the character of his laboratory did not prevent Frankel from devoting most of his time to research. Already in the very beginning he saw that training students in basic research was the best way to develop manpower of high quality which could tackle theoretical as well as applied problems. In this Frankel was successful, and his success became widely known. He quickly rose on the academic ladder, and he was soon given the opportunity to be independent and choose his own topics of research. Already in 1926 Frankel was appointed Instructor, Lecturer in 1934, Associate Professor in 1947, and Full Professor in 1953.

Very soon his laboratory on Mt. Scopus became a source of inspiration for students and young research workers, and many turned to him to learn from his knowledge, wisdom and experience. His department, then the Department of Theoretical Organic Chemistry and Macromolecular Chemistry, and now the Department of Organic Chemistry A, became well-known locally and abroad.

It is difficult to imagine the many obstacles which had to be overcome by the scholars who founded the University. The number of scientists was pitifully small and there was usually no one with whom to exchange thoughts on scientific work. There were practically no budgets for research and it was difficult to keep contact with the large scientific centres of Europe, England, and America. The belief in the increasing importance of science and technology, the hope that the Hebrew University would become a Jewish scientific national and international centre, and only the feeling that it would be impossible to run health services, schools, modern industry and agriculture without developed scientific centres motivated and stimulated the small and isolated group of scholars on Mt. Scopus. Whoever had the privelege of working with such scholars as Fodor, Adler, Picard, Fraenkel, Sambursky, Amira and the late Eig, Bodenheimer, Fekete, Moshe Weizmann, Reifenberg and others, could not but be infected by their enthusiasm and devotion. This wonderful group of pioneers and scholars laid the foundations for regional and general science in Israel, and for the Hebrew University of Jerusalem.

Among this group of pioneer scientists Frankel found friends close to him in thought and in action, and with them he worked to train a scientific staff of high calibre and to obtain the tools required for research and to establish contact with research workers abroad.

Frankel never tired in his struggle to improve research and teaching and never accepted compromises. Often, he made it difficult for those who did not understand him and his aims which sometimes seemed impossible, but even those who opposed him appreciated him and respected him for his honesty, modesty and fairness and often came to accept his plans.

His enthusiasm did not diminish even when he had to leave his laboratory on Mt. Scopus during the War of Liberation. With his assistants and students he rebuilt his laboratory in the Terra Sancta building, and within a short time succeeded in restoring the department, and turning once more into one of the most important and highly respected departments in the natural sciences.

Frankel always taught with dedication, and he always devoted much attention to the advancement of his own teaching programmes and to those of the University in general. His lectures in theoretical organic chemistry and macromolecular chemistry are among the best, excelling in their logical organization, and often also attended by students from other branches of science.

Professor Frankel is very meticulous and exacting in his own experimental work, and demands the same of his assistants and students whom he wishes to train in good technique as well as in self-criticism. In Frankel's laboratory, research has always been carried out in various fields of chemistry. His meticulousness and attention to detail never made him lose sight of general and basic problems, and never made him impose his will on his assistants and students. He gave his best students full academic freedom and extended to them the help needed to develop independent lines of research.

In spite of Frankel's many roles at the University, he devoted most of his time to creative research. The scientific works which he has published to date prove a true creative ability inspired by curiosity, broad horizons and deep knowledge, tempered by a well-developed sense of criticism and cold analytical logic. In his work, Frankel departed from the subjects accepted at the time in classical organic chemistry, and he chose modern subjects whose recent development proved the wisdom of his choice The macromolecular substances attracted him because of their theoretical and practical interest. Frankel clearly envisioned that the study of the structure and properties of the synthetic polymers would throw new light on the properties of important natural macromolecular substances such as the proteins and nucleic acids. Similarly, he anticipated that the synthetic macromolecular substances which are used in the plastic industry would play an important role in Israel. The mechanism of polymerization, and the investigation of the properties of macromolecular substances, became one of the basic subjects of his research. He used his broad knowledge in organic and in physical chemistry, and added to the subjects of his research, investigations in the field of theoretical organic chemistry, classical organic chemistry and colloidal chemistry.

Frankel was a pioneer in the development of methods for the preparation of high molecular peptides, the poly-α-amino acids. In recent years these polymers have become the most important synthetic models for proteins and natural peptides. In the field of polymerization of vinyl monomers he contributed much to the investigation of the mechanism of anionic polymerization and to the clarification of the mechanism of initiation of the polymerization by metal ketyls. The last works are among the most important in the field.

Frankel's works in colloidal chemistry and physical chemistry were carried out mostly when he first came to the University, and they contributed much to the understanding of the forces acting between amino acids and peptides in solution, and to the understanding of the colloidal properties of solutions of gelatin.

The physico-chemical research of the interaction between sugars and amino acids and peptides deserves special mention, since it sheds light on one of the important reactions between two biological components.

Among his many theoretical organic studies, his work on the photoisomerization in azo-compounds should be mentioned and also the work on the geometrical isomerism of azo-naphthalenes.

Finally, in order to complete the description of Frankel's versatility, we should mention the many investigations carried out in his laboratory on the classical syntheses of various compounds derived from amino acids, peptides, aliphatic and aromatic compounds, and also the interesting works on proteolytic enzymes and on the enzymatic properties of native papain.

The interest of Frankel in biological and chemical catalysts inspired him to write a book of wide scope and high quality on catalysis —"Katalytische-organische Arbeitsmethoden", which was published in the famous series of books on methods in biochemistry edited by E. Abderhalden, and which served for a long time as the reference book in the field of catalysis. Recently we have been enriched with another book by Frankel, together with S. Patai, "Tables for the Identification of Organic Compounds". Undoubtedly, this book too, will be of much value to chemists.

The creative ability of Prof. Frankel did not decrease through the years, and he continues steadily to contribute to science. The experience and knowledge which he gained through the years of his fruitful work serve him as stepping stones to conquer new and unknown fields. We extend our best wishes to him and hope that he will be able to teach and guide many generations of students and young research workers.

We are happy to celebrate the 60th birthday of Prof. Frankel — an excellent scientist and teacher, a good friend, and a modest and beloved man.

EPHRAIM KATCHALSKI

LIST OF PUBLICATIONS OF PROF. MAX FRANKEL

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ORGANIC FLUORINE COMPOUNDS. PART XXVI*. THE SYNTHESIS OF FLUORINE COMPOUNDS FROM SULPHONIC ACID ESTERS

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ABSTRACT

The conditions for the transformation of sulphonic acid esters into alkyl fluorides by reaction with potassium fluoride in diethyleneglycol have been studied systematically. By an application of the results to the *p*-toluenesulphonates of aldehyde cyanohydrins a new method for the synthesis of α-fluoronitriles (and α-fluoroacids) has been worked out. Comparative experiments with anions other than fluoride are reported.

The nucleophilic replacement of sulphonate ester groups by fluoride ions has been shown by Pattison and Millington[1] to be suitable for the transformation of alcohols into the corresponding fluorides. A systematic study of this method has been carried out, diethyleneglycol serving as solvent and potassium fluoride as nucleophilic reagent. Whilst the optimum conditions for the reaction are described in the experimental section of this paper, the following observations appear to deserve mention: Potassium fluoride causes to some extent a decomposition of diethyleneglycol into dioxan and water[2], and at the temperature of the reaction some potassium hydroxide is formed (by hydrolysis of potassium fluoride) which decomposes the sulphonates. It is therefore advantageous, to pass through the reaction mixture a current of hot dry air, even in the cases in which the fluoride is removed constantly from the reaction mixture. In Table I the alcohols are listed which give yields above and below 50% of the corresponding fluorine compounds, respectively. A number of alcohols could not be converted into the fluorides by this method: ethyl hydracrylate, diethyl tartrate, diethyl malate, benzyl alcohol, allyl alcohol, crotyl alcohol, β-methoxy-ethanol, β-ethoxy-ethanol, propyleneglycol, trimethyleneglycol, tetramethyleneglycol, and pentamethyleneglycol.

In one particular case, the method has proved of special importance, *viz.* in the preparation of α-fluorinated nitriles. In this case, the cyanohydrin of an aldehyde was esterified and the derivative — without isolation — heated with potassium fluoride:

$$R.CHO \longrightarrow R.CH(CN).OH \longrightarrow R.CH(CN).OSO_2.C_6H_5 \longrightarrow R.CHF.CN.$$

A number of examples are reported in the Experimental Part.

In order to avoid the difficulties caused in the reaction with potassium fluoride by the glycol serving as solvent, we have tried a modification of the reaction, which does

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not require a solvent and which, indeed, *cannot* be carried out in any solvent, *viz.* the use of the hydrofluoride of an amine instead of potassium fluoride. The analogous preparation of alkyl *chlorides* from sulphonates and trialkylammonium *chlorides* has been reported by Klamann[3]. Whilst tetramethyl, trimethylbenzyl and tri-(β-hydroxyethyl) ammonium fluoride were inactive and pyridinium and dimethylphenylammonium fluoride gave unsatisfactory results, good results were obtained with the hydrofluorides of triethylamine, trimethylamine, dicyclohexylamine, and especially of technical "trioctylamine". Table II gives the results obtained with "trioctylamine" hydrofluoride. In general the yields are lower than with potassium fluoride but in some cases replacement reactions could be carried out in which potassium fluoride failed. Again, negative results were obtained with benzyl alcohol, diethyl malate, dimethyl tartarte, ethyl glycerate and propyleneglycol.

It may be added that "trioctylamine" hydrofluoride converted ethyl chloroacetate and hexyl bromide into ethyl fluoroacetate and hexyl fluoride in yields of 21 and 30%, respectively.

For comparison, the reaction of alkyl *p*-toluenesulphonates with lithium chloride, sodium bromide, sodium iodide, potassium cyanide and potassium thiocayanate has been studied under the same conditions. The results are summarized in Table III. In one instance (*n*-hexyl *p*-toluenesulphonate), parallel experiments have been carried out with sodium, potassium and lithium chloride. The first reacted more sluggishly, the third more quickly than potassium chloride, but the yield was the same in all three cases. Sodium nitrite gave with *n*-octyl *p*-toluenesulphonate under the same conditions only very little of a nitrocompound and mainly octanoic acid.

A brief summary of some of these results has been published elsewhere[4].

EXPERIMENTAL

Preparation of the alkyl sulphonates

For the preparation of quantities larger than about 0.3 mole, the usual method[5], *viz.* the reaction between the alcohol and the sulphonyl chloride in pyridine, is not very practical. We prepared the sodium alkoxide from the alcohol with sodium ethoxide or sodium hydride in benzene or xylene; thus, quantitative yields of the esters were obtained, and the crude product resulting from the evaporation of the solvent *in vacuo* could be used directly.

(a) *With sodium ethoxide.* To a suspension of dry sodium ethoxide (1 mole) in 1 l. of xylene, the desired alcohol (1 mole) was added and the mixture heated with stirring in a column until the ethanol liberated and about 300 ml of xylene had distilled over. To the product, *p*-toluenesulphochloride (0.98 mole; 187 g) or benzenesulphonyl chloride (0.98 mole; 173 g) in 300 ml of xylene was added, the temperature being kept at 20°, for 30–40 mins., and the mass refluxed for 2–3 hours, until no more acid chloride could be detected in the supernatant liquid (precipitate with alcoholic silver nitrate). The reaction product was poured into cold water and the organic layer dried and concentrated, first under 30mm, then under 1mm pressure, at a temperature not exceeding 80°.

(b) *With sodium hydride.* The desired alcohol (1 mole) in 100ml of benzene was added, with stirring, to a suspension of sodium hydride (24 g; 1 mole) in 600 ml of benzene. The stirring was continued for 30 mins.; then the sulphochloride was added and the experiment performed as described above.

Transformation of alkyl sulphonates into fluorides by means of potassium fluoride. General procedure

In order to obtain anhydrous reaction conditions, it is recommended to mix the potassium fluoride with diethyleneglycol (for 0.5 mole, 150 ml and 45 g of KF, which was employed in an excess of 50%) and to heat the mixture for 30 minutes at 100–110° in a vacuum of 30 mm. The reaction mixture is heated with a strong burner on an asbestos net; a three-necked flask is employed, mounted with a very efficient strirrer, a bent condenser leading to an ice-cooled receiver, thermometer, dropping funnel and an inlet for hot air. The air is dried with conc. sulphuric acid and heated to a temperature of 100°.

Method A (for fluorides boiling below 150°; quantities up to 0.3 mole). The mixture of potassium fluoride, diethyleneglycol and the alkyl sulphonate was quickly heated until the reaction set in; then the temperature was cautiously raised to 150°. After 5 minutes at this temperature, the heating was interrupted and a current of hot air passed through the mass for 10 minutes. The total distillate was then diluted with chloroform or methylene chloride (except for fluorides boiling below 80°), washed with 10% hydrochloric acid and a saturated sodium hydrogen carbonate solution, dried and fractionated. If the distilled fluoride was still contaminated with small quantities of olefinic material, it was washed with a solution of bromine in aqueous sodium bromide and distilled again.

Method B (for fluorides boiling below 150°; quantities above 0.3 mole). The mixture of potassium fluoride and the glycol was heated at 140–145° in a current of hot air and the sulphonate added dropwise as fast as possible (it is advisable to use a flask which is not filled to more than 65% by the initial mixture). The current of air was maintained at a flask temperature of 150° for another 10 minutes and the work-up carried out as in Method A.

Method C (for fluorides boiling above 150°). The mixture of potassium fluoride, the glycol *and* the sulphonate was heated with vigorous agitation to a temperature (internal) of 130–135° and kept at this temperature for 30 minutes. The product was cooled as quickly as possible, poured into water and extracted with chloroform. The chloroform solution was washed with 10% hydrochloric acid, sodium bicarbonate solution and a solution of bromine in aqueous sodium bromide (as the olefins formed and the fluoride are likely to boil at about the same temperature), dried, concentrated and the residue distilled *in vacuo*.

The experiments are summarized in Table I.

NOTES: (TO TABLE I)

a) In larger batches, considerably smaller yields have been obtained.
b) Hoffmann, F.W., 1950, *J. Org. Chem.*, **15**, 425.
c) Desreux, V., 1934, *Chem. Abstr.*, **28**, 5805.
d) Swarts, F., 1922, *Chem. Abstr.*, **16**, 3062.
e) Pattison, F.L.M. and Norman, J.J., 1957, *J. Amer. Chem. Soc.*, **79**, 2311.
f) The pure *p*-toluenensulphonate was used; m.p. 49° (Marvel, C.S. and Sekera, V.C., 1955, *Org. Syntheses*, Coll. Vol. **3**, p. 366).
g) The compound was prepared according to Renoll, M. and Newman, M.S., 1955, *Org. Syntheses*, Coll. Vol. **3**, p. 502, and the pure *p*-toluenesulphonate was used, m.p. 49°. (Cf. Tipson, R.S., Clapp, M.A. and Cretcher, L.H., 1943, *J. Amer. Chem. Soc.*, **65**, 1092).
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m) The di–benzenesulphonate was used.
n) Cf. Midgley, Th. and Henne, A.L., US.. Patent 2, 192, 143 (*Chem. Abstr.*, 1940, **34**, 4391).
o) Preparation according to Hibbert, H. and Carter, N.M., 1928, *J. Amer. Chem. Soc.*, **50**, 3120.
p) Preparation according to Hill, H.S., Whelen, M.S. and Hibbert, H., 1928, *J. Amer. Chem. Soc.*, **50**, 2235.

Transformation of alkyl sulphonates into fluorides by means of "trioctylamine" hydrofluoride

"Trioctylamine" was neutralized (*p*H 6–7) by 40% aqueous hydrofluoric acid and the water removed in *vacuo* at as low a temperature as possible. The solid residue was filtered and dried in a dessicator. Its analysis pointed rather to the formula of octylamine hydrofluoride.

Anal. Calcd. for $C_8H_{20}FN$: F, 12.8; N, 9.4. Found: F, 12.5; N, 9.3.

A mixture of the hydrofluoride (0.2 mole) and the alkyl sulphonate (0.2 mole) was heated, with vigorous agitation, at 125–130° for 30 min. in a flask connected to a bent condenser. The distillate was dissolved in methylene chloride and the residue in the reaction vessel washed with the same solvent. The combined solutions were washed with 15% potassium bicarbonate solution, water, 20% hydrochloric acid and again water, dried and distilled in a short column.

The results are summarized in Table II.

The following amine hydrofluorides — prepared as above, but less well defined — gave also positive, though inferior results, *n*-hexyl *p*-toluenesulphonate serving as, substrate: pyridine — yield 20%, dimethylaniline 10%, triethylamine 29%, trimethylamine 29%, dicyclohexylamine 40%. "Trioctylamine" gave in this case a yield of 51%.

TABLE I

Reaction of alkyl p-toluenesulphonates with potassium fluoride in di-ethyleneglycol

Alcohol	Quantity (mole)	Method	Product (% calc. on the alcohol)	Yield	Identification
	a) *Yields* > 50%				
n-Pentanol	0.5	B	*n*-pentyl fluoride	82	b.p. 60-61° [b]
n Hexanol	0.2; 0.5; 1.0	B	*n*-hexyl fluoride	92; 91; 85	b.p. 91-91° [c]
n-Heptanol	0.5	B	*n*-heptyl fluoride	95	b.p. 117° [d]
n-Octanol	0.2; 0.5; 1.0; 2.0	A, B	*n*-octyl fluoride	92; 90; 62; 85	b.p. 147° [d] (*Anal.* Calcd. for $C_8H_{17}F$: C, 72.7; H, 12.9; F, 14.4. Found: C, 73.0; H, 23.2; F, 14.3).
Lauryl alcohol	0.1; 0.5	C	lauryl fluoride	66; 70	b.p. 105-106° (5 mm)[e] (*Anal.* Calcd. for $C_{12}H_{25}F$: F, 10.1. Found: F, 9.9).
Cetyl alcohol [f]	0.1; 0.2	C	cetyl fluoride	57; 62	b.p. 185° (26 mm)[e] (*Anal.* Calcd. for $C_{16}H_{33}F$: F, 7.8. Found: F, 7.6).
4-Hydroxymethyl-2,2-dimethyl-1,3-dioxolane [g]	0.2; 0.5; 1.0	A, B	4-fluoromethyl-2,2-dimethyl-1,3-dioxolane	85; 92; 82	b.p. 133-134° (*Anal.* Calcd. for $C_6H_{11}O_2F$: C, 53.7; H, 8.2; F, 14.2. Found: C, 53.4; H, 8.0; F, 14.0).
2-Ethyl-4-hydroxymethyl-2-methyl-1,3-dioxolane [h]	1.0; 1.5	B	2-ethyl-4-fluoromethyl 2-methyl-1,3-dioxolane	84	b.p. 146-147° [k]
Ethyl lactate [i]	0.15 [a]	A	ethyl α-fluoropropionate	75	b.p. 121–122° [k]
Ethyl glycolate	0.15 [a]	A	ethyl fluoroacetate	55	b.p. 117–118°
	b) *Yields* < 50%				
2-Octanol	0.5	B	2-octyl fluoride	35	b.p. 137-138° [d] (*Anal.* Calcd. for $C_8H_{17}F$: C, 72.7; H, 12.9; F, 14.4. Found: C, 72.9; H, 13.0; F, 14.3).
1,3-Dichloropropan-2-ol [l]	0.5	B	1,3-dichloro-2-fluoropropane	13	b.p. 126° (*Anal.* Calcd. for $C_3H_5Cl_2F$; C, 27.5; H, 3.8; F, 14.5. Found: C, 27.9; H, 3.8; F, 14.2). A small forerun, b.p. 111°, was 1,3-dichloro-propylene (*Anal.* Calcd. for $C_3H_4Cl_2$: C, 32.4; H, 3.6. Found: C, 32.1; H, 3.6).
Diethyleneglycol [m]	0.5	B	di-(β-fluoroethyl) ether	7	b.p. 124-125° [n] (*Anal.* Calcd. for $C_4H_8OF_2$: C, 43.6; H, 7.3; F, 34.5. Found: C, 43.4; H, 7.2; F, 34.2).
5-Hydroxy-1,3-dioxan [o]	0.5	B	5-fluoro-1,3-dioxan	7	b.p. 103-105° (*Anal.* Calcd. for $C_4H_7O_2F$: C, 45.3; H, 6.6; F, 18.0. Found: C, 45.6; H, 6.8; F, 17.5).
4-Hydroxymethyl-1,3-dioxolan [o]	0.5	B	4-fluoromethyl-1,3-dioxolan	8	b.p. 111-112° (*Anal.* Calcd. for $C_4H_7O_2F$ C, 45.3; H, 6.6; F, 18.0 Found: C, 45 1; H, 6.7; F, 17.6).
5-Hydroxy-2-phenyl-1,3-dioxan [p]	0.25	C	5-fluoro-2-phenyl-1,3-dioxan	5	b.p. 117-119° (30 mm). (*Anal.* Calcd for $C_{10}H_{11}O_2F$: C, 65.9; H, 6.0; F, 10.4. Found: C, 66.2; H, 5.7; F, 10.8).
4-Hydroxymethyl-2-phenyl-1,3-dioxolan [p]	0.25	C	4-fluoromethyl-2-phenyl-1,3-dioxolan	10	b.p. 111-114° (30 mm). (*Anal.* Calcd. for $C_{10}H_{11}O_2F$: C, 65.9; H, 6.0 F, 10.4. Found: C, 65.7; H, 5.8; F, 10.0).

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TABLE II

Reaction of alkyl p-*toluenesulphonates with "trioctylammonium" fluoride.*

Alcohol	Quantity (mole)	Product	Yield (calc. on the alcohol)
n-Pentanol	0.15	*n*-Pentyl fluoride, b.p. 60–61°	52
n-Hexanol	0.20	*n*-Hexyl fluoride, b.p. 90–91°	51
n-Octanol	0.15	*n*-Octyl fluoride, b.p. 140–141°	35
2-Ethoxyethanol	0.15	Ethyl (β-fluoroethyl) ether, b.p. 74° a)	22
Diethyleneglycol	0.10	Di-(β-fluoroethyl) ether, b.p. 123–124°	20
Trimethyleneglycol	0.30	1,3-Difluoropropane, b.p. 40–41° c)	23
Tetramethyleneglycol	0.30	1,4-Difluorobutane, b.p. 78° d)	25
Pentamethyleneglycol	0.30	1,5-Difluoropentane, b.p. 104° d)	17
Ethyl glycolate	0.25	Ethyl fluoroacetate, b.p. 117–118°	32
Ethyl lactate	0.15	Ethyl α-fluoropropionate b.p. 121–122°	42

Notes: a) Gryszkiewicz–Trochimowski, E., Sporzynski, A. and Wnuk, J., 1947, *Rec. Trav. Chim.* **66**, 413, 502.
b) Midgley, Th. and Henne, A.L., U.S. Patent 2, 192, 143, (*Chem. Abstr.*, 1940, **34**, 4391,
c) Hoffmann, F.W., 1950, *J. Org. Chem.*, **15**, 425.
d) Hoffmann, F.W., 1949, *J. Org. Chem.*, **14**, 105.

α-Fluoronitriles from aldehydes

General Procedure. The freshly distilled aldehyde (0.6 mole) was added, with cooling (5–15°) and stirring, to 25 g of sodium cyanide or 35 g of potassium cyanide, dissolved in a minimum quantity of water; then a solution of benzenesulphonyl chloride (0.5 mole) in methylene chloride (120 ml) was added with vigorous stirring at 15° (*p*-toluenesulphonyl-chloride which may be used instead, has the disadvantage that it tends to crystallize out). After one hour at 15° and six hours at 25° (the aqueous phase must be neutral; otherwise, the stirring is continued at the latter temperature), the organic layer was separated, washed with water, sodium bisulphite solution (until the organic layer is free of aldehydes; test with 2,4-dinitrophenylhydrazine reagent) and again with water, dried with magnesium sulphate and concentrated under 30 mm pressure at a temperature not exceeding 60°. The residue (mainly the benzenesulphonate of the cyanohydrin) was then dissolved in diethyleneglycol and treated with potassium fluoride, as described above (method B). It is not advisable to work with batches larger than 0.5 mole.

Fluoroacetonitrile. The product (yield 30%, 9 g) boiled at 80° and was identified by hydrolysis to fluoroacetic acid and conversion of the latter to the amide; m.p. 108°[7]. The distillation residue was a dark resin, which contained fluorine and nitrogen, but no sulfur.

α-Fluoropropionitrile. The product (yield, 52%; 19 g) boiled at 95–96°. *Anal.* Calcd. for C_3H_4FN: C, 49.3; H, 5.5; F, 26.0; N, 19.2. Found: C, 49.5; H, 5.5; F, 25.7; N, 19.0.

Hydrolysis gave α-fluoropropionic acid, b.p. 85° (25 mm)[8], identified as the amide of m.p. 76°[8].

a-Fluorobutyronitrile, b.p. 115–116°; yield, 25 g (58%) *Anal.* Calcd. for C_4H_6FN: C, 55.2; H, 6.9; F, 21.8; N, 16.1 Found: C, 55.4; H, 7.0; F, 21.6; N, 16.0.

a-Fluorovaleronitrile, b.p. 137–138°; yield, 25 g (49%). *Anal.* Calcd. for C_5H_8FN C, 59.4; H, 7.9; F, 18.8; N, 13.9: Found: C, 59.3; H, 8.0; *F* 18.6; N, 13.7.

a-Fluoroisovaleronitrile, from isobutyraldehyde, b.p. 126–127°; yield, 30 g (60%). *Anal.* Calcd. for C_5H_8FN: C, 59.4; H, 7.9; F, 18.8; N, 13.9. Found: C, 59.5; H, 8.2; 18.7; N, 13.8.

a-Fluoroisocapronitrile, from isovaleraldehyde, b.p. 132–133°, yield, 33 g (57%). *Anal.* Calcd. for $C_6H_{10}FN$: C, 62.6; H, 8.7; F, 16.5; N, 12,2. Found: C, 62.6; H, 9.0; F, 16.2; N, 11.8.

Benzaldehyde, phenylacetaldehyde, acrolein and crotonaldehyde gave either no sulphonates at all or unstable sulphonates.

Other nucleophilic reactions of alkyl p-*toluenesulphonates. General procedure*

The reactions were carried out as in the case of the fluorides; the inorganic salts (LiCl, NaBr, NaI, KCNS) with the exception of potassium cyanide were dried at 110° for 5 hours. For each tenth of a mole of the sulphonic acid ester, 50 ml of diethyleneglycol and 0.12 mole of the inorganic salts were employed. In the case of the chloride, bromide and cyanide, the three components were heated, with vigorous agitation, at 135–145° (internal temperature); in the case of the iodide and thiocyanate, the sulphonic acid ester was added slowly from a dropping funnel, care being taken that the exothermic reaction did not exceed the temperature of 145°.

If the product was volatile, it distilled off within a very short time; the reaction was brought to completion by additional heatng for 10 min. If the product did not distil, the reaction mixture was heated for the following periods of time: chlorides 150 min.; cyanides, 1 hr.; bromides, 45 min.; iodides and thiocyanates, 10 min.

The reaction mixture was cooled, diluted with three volumes of water and extracted with methylene chloride (except in the case of butyl chloride). The extract was combined with the distillate, if any, and washed with water and sodium bicarbonate solution. In the case of the iodides, the solutions were also washed with sodium thiosulphate solution and in the case of the nitriles with conc. hydrochloric acid and then with water.

After drying, the methylene chloride was distilled off in a column and the residue fractionated.

The results are listed in Table III.

TABLE III

Reactions of alkyl p-*toluenenesulphonates with various salts*

Alkyl	Salt	Quantity (mole)	Product	Yield [a)] %	Remarks
n-Butyl	LiCl	0.5	C_4H_9Cl; b.p. 76–77°	92	b
	NaBr	0.25	C_4H_9Br; b.p. 102–103°	86	c
	NaI	0.25	C_4H_9I; b.p. 127–128°	72	d
	KCN	0.5	C_4H_9CN; b.p. 138–139°	74	e
	KSCN	0.3	C_4H_9SCN; b.p. 179–81°	97	f
n-Hexyl	LiCl	0.5	$C_6H_{13}Cl$; b.p. 132–133°	92	
	NaBr	0.2	$C_6H_{13}Br$; b.p. 155°	90	
	NaI	0.2	$C_6H_{13}I$; b.p. 176–178°	76	
	KCN	0.5	$C_6H_{13}CN$; b.p. 179–180°	77	
	KSCN	0.25	$C_6H_{13}SCN$; b.p. 98–99° (22 mm)	98	
n-Octyl	LiCl	0.2	$C_8H_{17}Cl$; b.p. 180–181°	89	
	NaI	0.2	$C_8H_{17}I$; b.p. 129–132° (21mm)	75	
	KCN	0.3	$C_8H_{17}CN$; b.p. 73–74° (22mm)	81	
	KSCN	0.2	$C_8H_{17}SCN$; b.p. 134–136° (22mm)	95	g
Ethyl	NaI	0.5	C_2H_5I; b.p. 70–72°	96	h

a) Yield, calculated on the *alcohol*.

b) Drahowzal, F. and Klamann, D., 1951, Monatsh., **82**, 970, report yields of 10, 13.5 and 59% for sodium chloride, potassium chloride and HCl, under different conditions.

c) According to ref. 5, 15%

d) According to ref. 5, 78.5%

e) According to ref. 5, 38.6%

f) According to ref. 5, 86% (in ethanol 68%)

g) According to ref. 5, 83%

h) According to ref. 5, 82%.

ACKNOWLEDGMENT

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1,5-DIPHENYLBIURET, 1,7-DIPHENYLTRIURET AND RELATED COMPOUNDS, THEIR PREPARATION AND U.V. ABSORPTION SPECTRA

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ABSTRACT

A series of compounds of the general formula $Ph(NHCO)_nPh$(II) and $Ph(NHCO)_nNHPh$ (III) have been prepared and their U.V. absorption spectra in neutral, acidic and basic media examined. Some interpretations of the spectra observed, have been suggested.

INTRODUCTION

In a previous study[1] a number of benzoylpolyglycineanilides (I), $PhCO(NHCH_2CO)_n$ NHPh (n=1–6), have been prepared and their U.V. spectra examined. It was found that these compounds exhibit a hypsochromic shift with increasing chain length. It was deemed interesting to synthesize a series of compounds of the general formula $Ph(NHCO)_nPh$ (II) and $Ph(NHCO)_nNHPh$ (III) in order to determine whether these compounds, that lack the central methylene group of (I), behave similarly.

1,5-Diphenylbiuret (III, n=2) was prepared according to the literature by heating either phenyl isocyanate with phenylurea[2], or ethyl allophanate with aniline[3], though at a somewhat higher temperature than that reported[4]. When phenylurea and phenyl isocyanate were allowed to react in an inert solvent such as toluene, the asymmetrical 1,3-diphenylbiuret was obtained. 1-Benzoyl-3-phenylurea (II, n=2) was prepared from benzamide and phenyl isocyanate, 1-benzoyl-5-phenylbiuret (II, n=3) from benzoyl chloride and phenylbiuret, which in turn was obtained from urea and phenyl isocyanate[5,6]. 1,7-Diphenyltriuret (III, n=3) could not be obtained from two equivalents of phenyl isocyanate and one equivalent of urea, or from phenylbiuret and phenyl isocyanate, but the reaction of two equivalents of phenyl carbamidchloride[7] with one equivalent of urea was successful. 1-Methyl-1,3-diphenylurea was obtained from N-methylaniline and phenyl isocyanate. The yields in all these preparations were relatively high (65–85%) and no systematic attempts were made to improve them.

EXPERIMENTAL

(All melting points reported are uncorrected).

1,5-*Diphenylbiuret* (*III*, *n*=2)[4]

A mixture of 14g of ethyl allophanate and 20g of aniline was refluxed for 1/2 hour.

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The ethyl allophanate dissolved, and 1,5-diphenylbiuret crystallized upon cooling. It was filtered, washed with a little cold ethanol and recrystallized from ethanol or acetonitrile: it melted at 224° (lit.[4]:210°).

Anal. Calcd. for $C_{14}H_{13}N_3O_2$: C, 65.9: H, 5.1: N, 16.4
Found : C, 66.2: H, 5.1: N, 16.6.

1,3-*Diphenylbiuret*

A mixture of 5g of phenylurea, 4.5g of phenyl isocyanate and 250ml of dry toluene was refluxed with stirring until a clear solution resulted. After further 20 minutes of heating, the solution was kept overnight and the 1,3-diphenylbiuret filtered and recrystallized from ethanol or acetonitrile: m.p. 171° (lit.[3]: 168°).

Anal: Calcd. for $C_{14}H_{13}N_3O_2$: C, 65.9: H, 5.1: N, 16.4.
Found : C, 65.7: H, 5.3: N, 16.3.

1-*Benzoyl-3-phenylurea* (*II*, *n*=2)

A mixture of 3g of benzamide and 2g of phenyl isocyanate was heated with stirring at 130^{0}, until the product was a completely dry mass. It was then recrystallized several times from ethanol and melted at 214°.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 70.0: H, 5.0: N, 11.6.
Found : C, 70.4; H, 4.7: N, 11.3.

Phenylbiuret

This compound was prepared according to Lakra and Dains[6] and melted at 172° (lit.[5]: 150°–167°) after recrystallization from methanol.

Anal. Calcd. for $C_8H_9N_3O_2$: C, 53.6: H, 5.0: N, 23.4.
Found : C, 53.5: H. 5.2: N, 23.4.

1-*Benzoyl-5-phenylbiuret* (*II* *n*=3)

Equimolar quantities of phenylbiuret and benzoyl chloride were heated on a boiling water bath under exclusion of moisture. The phenylbiuret dissolved quickly, but heating was continued until a dry mass was formed. The product was recrystallized several times from glacial acetic acid: m.p. 256°.

Anal. Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.6: H, 4.6: N, 14.8.
Found : C, 63.8: H, 4.9: N, 15.3.

1,7-*Diphenyltriuret* (*III*, *n*=3)

Dry hydrogen chloride was bubbled at 0° through 10g of phenyl isocayanate, until the liquid turned completely solid[7]. After addition of 2.5g of urea the mixture was heated at 90° with stirring. A clear solution resulted which turned gradually solid in the course of twenty minutes. Heating was continued until the solid was completely dry. It was then recrystallized from acetonitrile or glacial acetic acid and melted at 226°.

Anal. Calcd. for $C_{15}H_{14}N_4O_3$: C, 60.4: H, 4.7: N, 18.8.
Found : C, 60.6: H, 4.7: N, 18.5.

1-*Methyl*-1,3-*diphenylurea*

Equimolar quantities of N-methylaniline and phenyl isocyanate were heated with stirring at 90°–100° in a stream of nitrogen until the mixture turned solid (5–10 minutes). The product was recrystallized from ethanol: m.p. 95°.

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 74.4: H, 6.2: N, 12.4.
Found : C, 74.4: H, 6.2: N, 11.9.

1,3-*Quinazolinedione* (*benzoyleneurea*)[8]

This compound was prepared for comparison and is highly hydroscopic. It was recrystallized from glacial acetic acid.

Anal. Calcd. for $C_8H_6N_2O_2$: C, 59.2: H, 3,7.
Found : C, 59.4: H, 3.7.

MEASUREMENTS

For the determination of the U.V. spectra (Beckman DV spectrophotometer), spectroscopically pure ethanol was employed. For basic and acidic media, a 1% (w/v) solution of sodium in ethanol and a 1N solution of 70% perchloric were used, respectively. The spectra are summarized in Figures 1–5. Figures 1 and 2 show the spectra of compounds of type III and II, respectively, in neutral ethanol, Figures 4 and 5 those in basic medium, and Figure 3 shows the spectra of monophenylurea and monophenylbiuret in neutral ethanol. In basic medium, the lower wave length limit at which measurements could still be carried out is 230 mμ: beyond this wave length, both reference and sample solutions turned opaque. The absorption spectra of the compounds in acidic medium have not been reproduced, as they are identical with those in neutral medium.

DISCUSSION[9]

An examination of the U.V. spectra shows certain similarities to those of the benzoypolyglycineanilides (I) reported previously[1]. In the series 1,3-diphenylurea (III, n=1) (257.5 mμ), 1,5–diphenylbiuret (III, n=2) (250 mμ), 1,7-diphenyltriuret (III, n=3) 247.4 mμ) a hypsochromic shift is observed which, however, becomes smaller on ascending the series, whereas the shift in the benzoylpolyglycineanilides (I) shows a constant difference of 3 mμ/ unit. In comparison, the monophenyl compounds (Figure 3) absorb at 235 mμ (phenylurea) and 237 mμ (phenylbiuret) with no indication of a hypsochromic shift, whilst urea and biuret do not show any absorption in this region[10,11].

The compounds (II) exhibit a somewhat different pattern. The cyclic 1,3-quinazolinedione (benzoyleneurea) (Figure 2) shows a second absorption band at 310 mμ

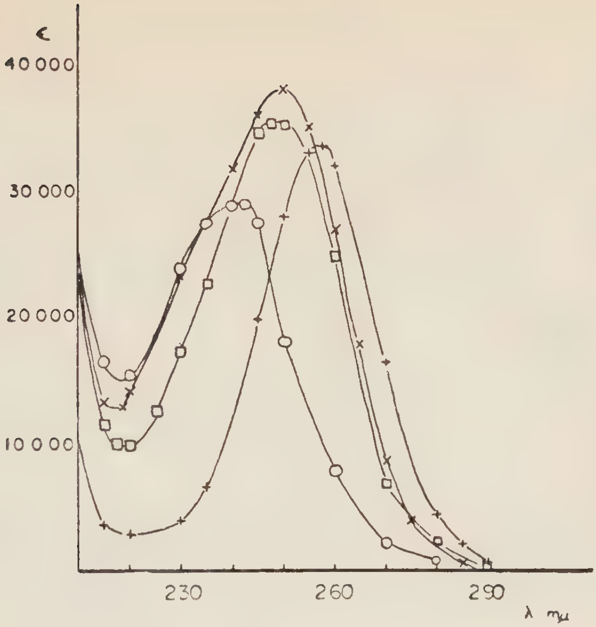


Figure 1
Spectra of compounds III in neutral ethanol:
+ 1,3-diphenylurea (n=1),
× 1,5-diphenylbiuret (n=2),
□ 1,7-diphenyltriuret (n=3),
○ 1,3-diphenylbiuret

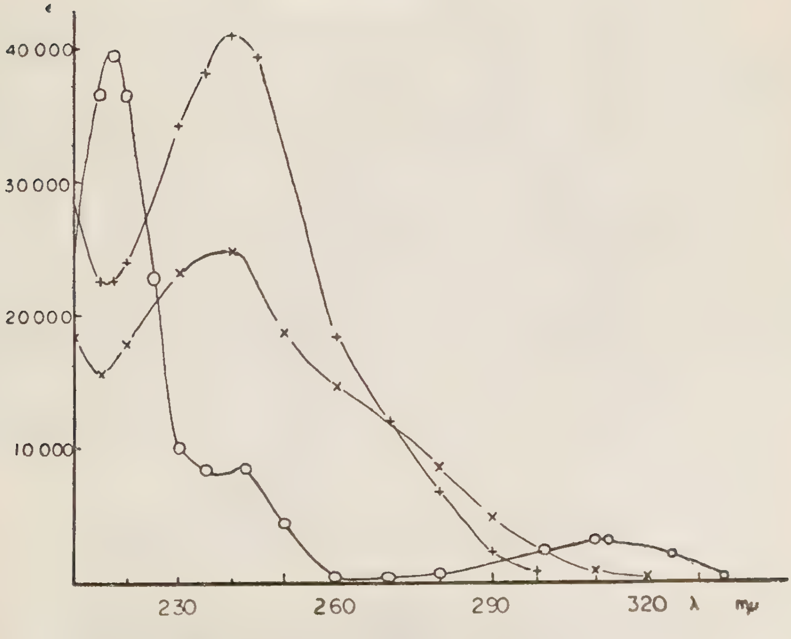


Figure 2
Spectra of compounds II in neutral ethanol:
○ 1,3-quinazolinedione,
× 1-benzoyl-3-phenylurea (n=2),
+ 1-benzoyl-5-phenylbiuret (n=3)

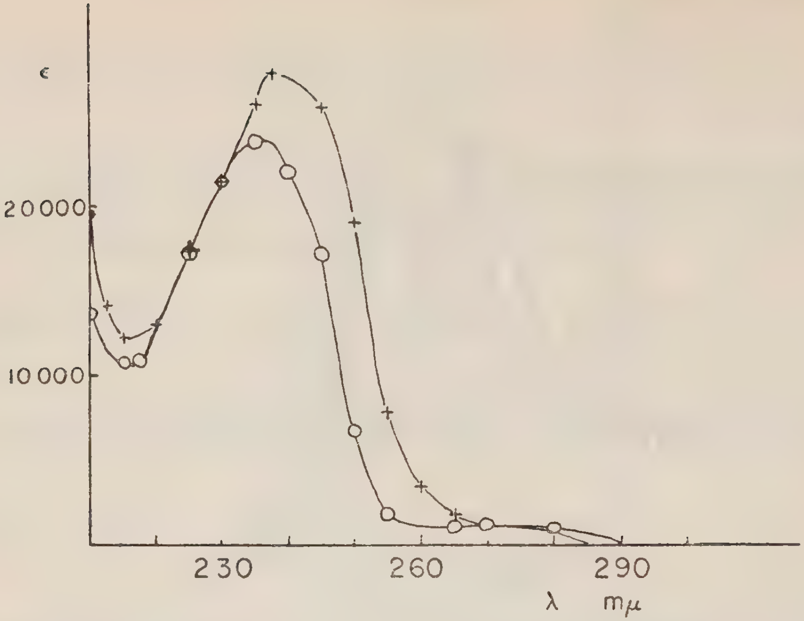


Figure 3
Spectra of ○ phenylurea,
+ phenylbiuret in neutral ethanol

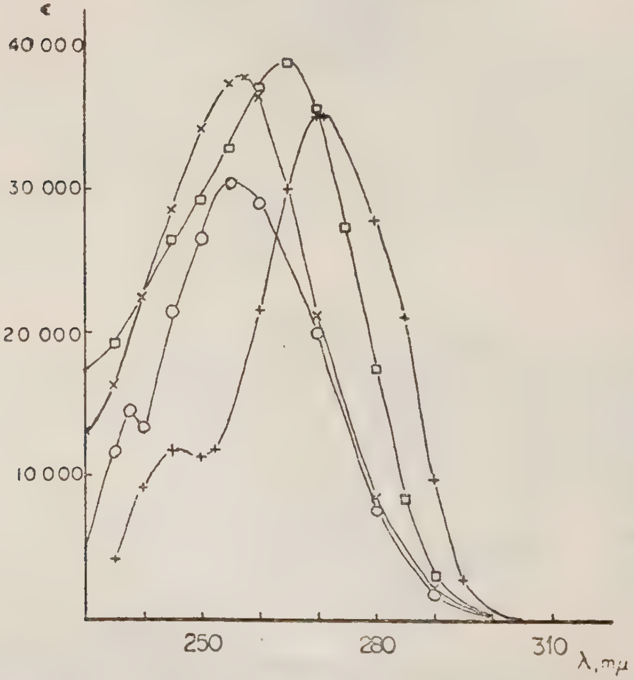


Figure 4
Spectra of compounds III in a 1% (w/v) solution of Na in ethanol
○ 1,3-diphenylurea (n=1).
+ 1,5-diphenylbiuret (n=2),
□ 1,7-diphenyltriuret (n=3),
× 1,3-diphenylbiuret

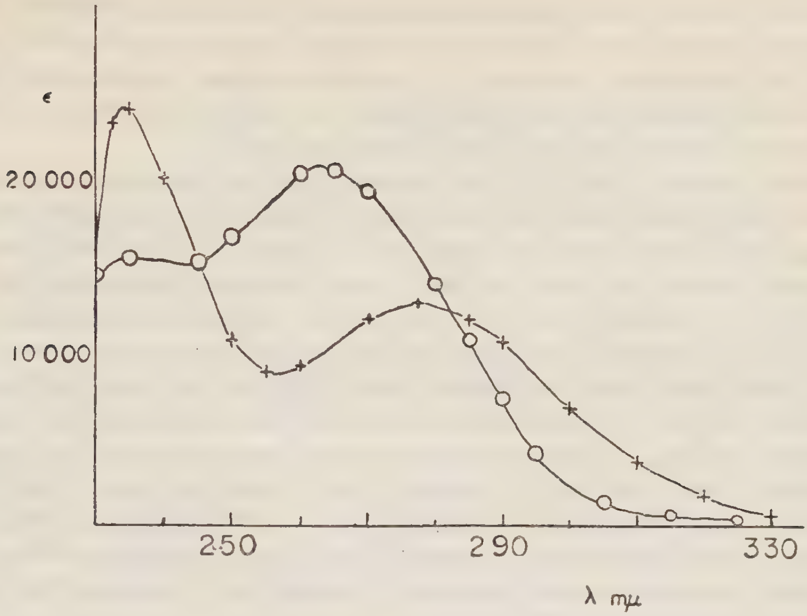
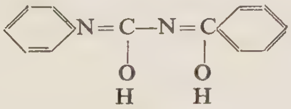


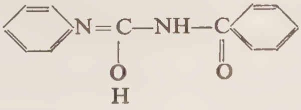
Figure 5
Spectra of compounds II in a 1% (w/v) solution of Na in ethanol
+ 1-benzoyl-3-phenylurea (n=2),
○ 1-benzoyl-5-phenylbiuret (n=3)

which can be ascribed to the lactim form. Indeed, this compound is a strong acid[8] and the general shape of its absorption curve is similar to that of α-naphthol[12].

This second absorption band at 310 mμ is missing in the other compounds of type (II). 1-Benzoyl-3-phenylurea (II, n=2), which is similar to benzoyleneurea except for the fact that its CONH chain connects not two points at the same phenyl group but two different phenyl groups, shows in addition to its main band at 240 mμ a considerable absorption intensity at 275 mμ (ε=10,000). It may be that at least one of the two CONH groups in the chain exists in the lactim form which in conjugation with the neighbouring phenyl group could explain this absorption. The same may be true of 1-benzoyl-5-phenylbiuret (II, n=3) although the absorption intensity of this compound in the 280 mμ region is reduced (ε=6,000). The fact that the compounds of type II show pronounced absorption at a longer wave length than 240 mμ might indicate that conjugated formulae of the type



and



make a greater contribution to the structure of the molecule than in compounds of type III.

In acidic medium, the absorption spectra of all compounds studied are similar to those in neutral medium. This is unexpected since it is known that in acidic medium the CONH group binds protons[1,13]. It should be pointed out, however, that all compounds studied are insoluble in strong acids, so that the acidic ethanolic solution employed might have been too dilute to show any effect.

In basic medium (Figures 4, 5), all substances with the exception of 1,3-diphenylurea have their main absorption peak shifted to the red. This shift is of the order of magnitude of 20 mμ and may be due to the presence of an increased amount of the lactim form or its anion In three cases (1-benzoyl-3-phenylurea (II, n=2) (Figure 5), 1,3–diphenylurea (III, n=1) and 1,5-diphenylbiuret (III, n=2) (Figure 4) and additional absorption peak at a shorter wave length appears.

One might have expected 1,3-diphenylbiuret (Figure 1) to show an absorption spectrum composed of those of 1,3-diphenylurea and phenylurea. This however, is not the case, and 1,3-diphenylbiuret shows an absorption spectrum (240 mμ, ε=20,000) similar to that the 1,5-isomer. This is actually the largest hypsochromic shift in the series and exceeds even that found for 1,7-diphenyltriuret (III, n=3). A quite similar spectrum was found for 1-m-ethyll, 3-diphenylurea (240 mμ, ε=22,000) resembling the shift observed in passing from benzamide (225 mμ) to N,N-dimethylbenzamide (218 mμ)[13].

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N-ARYL-α- AND β-ASPARAGINES

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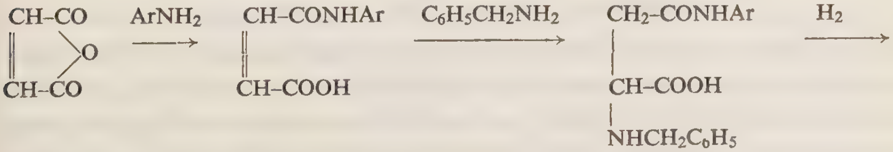
ABSTRACT

N-Aryl-β asparagines have been prepared by reaction of benzylamine with aryl-maleamic acids and subsequent hydrogenolysis of the benzyl group. The corresponding α-isomers have been obtained by reacting the mixed anhydride of N-benzyl-DL-aspartic acid and chloroformic acid with a large excess of the arylamines, equimolar quantities leading to β-asparagines.

N-Aryl-α and β-asparagines have been synthesised by means of the reactions employed for the preparation of N-alkyl-asparagines[1]. When aryl-amaleamic acids are refluxed with benzylamine in dioxane solution, N^2-benzyl-N-aryl-β-asparagines result which are then debenzylated by catalytic hydrogenolysis.

For the preparation of the α-isomers, the original procedure had to be modified, since the reaction of merely equimolar amounts of arylamines with the mixed anhydride of N-benzyl-DL-aspartic acid and chloroformic acid yielded β-amides in contradistinction to aliphatic amines which form α-amides under the same conditions On using, however, a large excess of the arylamines[2]. N^2-benzyl-N-aryl-α-asparagines were obtained together with the corresponding arylamine hydrochlorides. Separation from the latter proved difficult and yields were accordingly considerably lower than in the alkyl-asparagine series. N-Aryl-α-asparagines resulting after hydrogenolysis of the N^2-benzyl derivatives may be distinguished from the corresponding β-isomers by a positive biuret reaction[3] which in the case of phenyl- and β-naphtyl- α-asparagines gave a yellow instead of the normal violet colour. They can, moreover, be differentiated from α-asparagines by paper chromatography, since the latter, after treatment with cupric ion form complexes which prevent the development of ninhydrin colours on spraying with this reagent, whereas α-asparagines, possessing a free β-carboxyl group, are not affected by cupric ion and the colour reaction is not suppressed[4]. N^2-Benzyl-N-aryl-β-asparagines have higher melting points than the corresponding α-asparagine derivatives, and this is also true for the free asparagines, obtained after hydrogenolysis, although the difference is less pronounced.



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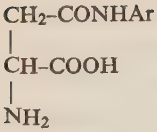
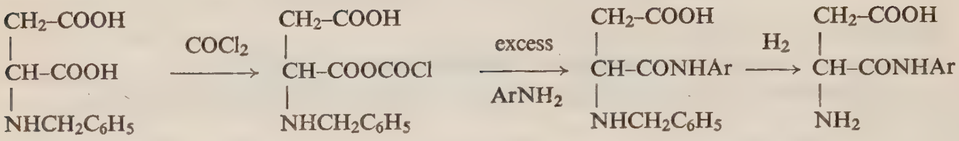


$$\begin{array}{l} CH_2\text{–}CONHAr \\ | \\ CH\text{–}COOH \\ | \\ NH_2 \end{array}$$



$$\begin{array}{l} CH_2\text{–}COOH \\ | \\ CH\text{–}COOH \\ | \\ NHCH_2C_6H_5 \end{array} \xrightarrow{COCl_2} \begin{array}{l} CH_2\text{–}COOH \\ | \\ CH\text{–}COOCOCl \\ | \\ NHCH_2C_6H_5 \end{array} \xrightarrow[ArNH_2]{excess} \begin{array}{l} CH_2\text{–}COOH \\ | \\ CH\text{–}CONHAr \\ | \\ NHCH_2C_6H_5 \end{array} \xrightarrow{H_2} \begin{array}{l} CH_2\text{–}COOH \\ | \\ CH\text{–}CONHAr \\ | \\ NH_2 \end{array}$$

EXPERIMENTAL

Micro-combustion analyses were made by Drs. Weiler and Strauss. Melting points were determined in a Fisher-Johns apparatus.

All substances prepared are listed in Tables I–V.

Preparation of N-aryl-maleamic acids

A solution of maleic anhydride (0.1 mole) in 150 ml of dry ether was cooled in an ice-bath and the arylamine (0.1 mole) in 20 ml of ether added with stirring. The N-aryl-maleamic acid which precipitated at once, was filtered off and washed with ether.

TABLE I

Preparation of N-aryl-maleamic acids

Substances were recrystallized from ethanol if not indicated otherwise

Substance, N-aryl-maleamic acid	Yield %	M.p. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
Phenyl	32	212	$C_{10}H_9O_3N$					7.3	7.2
o-Tolyl (*a*)	57	117	$C_{11}H_{11}O_3N$	64.4	65.0	5.4	5.1	6.8	6.7
m-Tolyl	78	170	$C_{11}H_{11}O_3N$	64.4	64.2	5.4	5.4	6.8	6.9
p-Anisyl	95	183	$C_{11}H_{11}O_4N$					6.3	6.0
o-Carboxyphenyl	Quant.	192	$C_{11}H_9O_5N$					5.9	5.4
p-Carboxyphenyl	94	230	$C_{11}H_9O_5N$	56.2	56.3	3.8	3.7	5.9	5.8
β-Naphthyl	76	198	$C_{14}H_{11}O_3N$					5.9	5.2

(*a*) Recrystallized from petroleum ether-acetone.

Preparation of N^2-benzyl-N-aryl-DL-β-asparagines

A mixture of the N-aryl-maleamic acid (0.026 mole) and benzylamine (0.026 mole) in 100–150 ml of dry dioxane was heated under reflux. After about 30 minutes the N^2-benzyl-N-aryl-asparagines started to settle in the reaction mixture and heating was continued for an additional period (from one to three hours). After cooling the precipitate was filtered and washed with acetone.

TABLE II

Preparation of N^2-benzyl-N-aryl-DL-β-asparagines

All substances were recrystallized from ethanol

Substance, N^2-benzyl-N-aryl-asparagine	Yield %	M.p. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
Phenyl	34	228	$C_{17}H_{18}O_3N_2$	68.4	68.2	6.0	5.8	9.4	9.3
o-Tolyl	38	225	$C_{18}H_{20}O_3N_2$	69.2	70.0	6.4	6.2	9.0	8.5
m-Tolyl	45	227	$C_{18}H_{20}O_3N_2$	69.2	69.2	6.4	6.6	9.0	8.9
p-Anisyl	63	226	$C_{18}H_{20}O_4N_2$	65.5	66.0	6.1	6.0	8.5	8.4
o-Carboxyphenyl	29	242	$C_{18}H_{18}O_5N_2$	63.2	63.5	5.3	5.3	8.2	8.0
p-Carboxyphenyl	27	245	$C_{18}H_{18}O_5N_2$	63.2	63.3	5.3	5.3	8.2	8.2
β-Naphthyl	65	233	$C_{21}H_{20}O_3N_2$	72.5	72.3	5.7	5.8	8.0	7.8

Preparation of N-aryl-DL-β-asparagines

The N^2-benzyl-N-aryl-asparagine (0.015 mole) was dissolved in 75 ml of glacial acetic acid and 0.2g of palladium chloride on charcoal catalyst (30%) added. Hydrogenolysis was carried out in a Parr low pressure apparatus for about five hours, the reaction bottle being heated to about 70°C by an infra-red lamp. After cooling, the catalyst was removed by filtration and the solvent evaporated in vacuo. The residue was recrystallized from water or ethanol.

TABLE III

Preparation of N-Aryl-DL-β-asparagines

Substances were recrystallized from ethanol if not indicated otherwise. Yields were almost quantitative

Substance, N-Aryl-asparagine	M.p. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
Phenyl (*a*)	255	$C_{10}H_{12}O_3N_2$	57.7	56.9	5.7	5.8	13.5	13.1
o-Tolyl	236	$C_{11}H_{14}O_3N_2$ $+ 1/2H_2O$	57.2	57.8	6.5	6.2	12.1	12.3
m-Tolyl	258	$C_{11}H_{14}O_3N_2$	59.4	59.5	6.3	6.3	12.6	12.5
p-Anisyl (*a*)	265	$C_{11}H_{14}O_4N_2$	55.5	55.7	5.9	5.9	11.8	11.6
o-Carboxyphenyl	245	$C_{11}H_{12}O_5N_2$ $+ 1/2H_2O$	50.6	50.2	5.0	4.7	10.7	10.7
p-Carboxyphenyl	255	$C_{11}H_{12}O_5N_2$ $+ H_2O$	49.0	49.2	5.2	5.0	10.3	9.7
β-Naphthyl (*a*)	267	$C_{14}H_{14}O_3N_2$	65.1	65.4	5.4	5.7	10.8	10.7

(*a*) Recrystallized from water.

Preparation of N^2-benzyl-N-aryl-DL-α-asparagines

The arylamine (0.1 mole) was added to a cooled solution of the mixed anhydride of N-benzyl-DL-aspartic acid and chloroformic acid[3], (freshly prepared from 4g of N-benzyl-DL-aspartic acid in 70 ml of dry dioxane), and left overnight at room temperature. (The phosgene used should be of high quality). The mixture of the amide and amine hydrochloride was filtered off and dried. Isolation of the former was affected by several recrystallizations from ethanol. The dioxane solution, which contained mostly uncontaminated amide, in small amount, was evaporated in vacuo to dryness and the residue recrystallized from ethanol.

TABLE IV

*Preparation of N^2-benzyl-N-aryl-*DL*-α-asparagines*
All substances were recrystallized from ethanol

Substance, N^2-benzyl-N-aryl-α- asparagine	Yield %	M.P. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
Phenyl	23	188	$C_{17}H_{18}O_3N_2$	68.4	68.4	6.0	5.9	9.4	9.2
o-Tolyl	15	190	$C_{18}H_{20}O_3N_2$	69.2	69.3	6.4	6.3	9.0	8.9
m-Tolyl	35	176	$C_{18}H_{20}O_3N_2$	69.2	68.5	6.4	6.7	9.0	8.9
p-Anisyl	8	187	$C_{18}H_{20}O_4N_2$	65.5	65.5	6.1	6.1	8.5	8.5
o-Carboxyphenyl	19	185	$C_{18}H_{18}O_5N_2$	63.2	63.5	5.3	5.3	8.2	8.0
p-Carboxyphenyl	20	225	$C_{10}H_{18}O_5N_2$	63.2	63.0	5.3	5.3	8.2	8.5
α-Naphthyl	32	186	$C_{21}H_{20}O_3N_2$	72.5	71.5	5.7	5.7	8.0	7.8
β-Naphthyl	24	213	$C_{21}H_{20}O_3N_2$	72.5	72.7	5.7	5.7	8.0	7.8

*Preparation of N-aryl-*DL*-α–asparagines*

The procedure is the same as for the β-isomers.

TABLE V

*Preparation of N-aryl-*DL*-α-asparagines. All substances were recrystallized from ethanol. Yields were almost quantitative*

Substance, N-aryl-α-asparagine	M.P. °C	Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
Phenyl	242	$C_{10}H_{12}O_3N_2$	57.7	57.9	5.7	6.0	13.5	13.1
o-Tolyl	220	$C_{11}H_{14}O_3N_2$	59.4	58.5	6.3	6.3	12.6	12.6
m-Tolyl	238	$C_{11}H_{14}O_3N_2$	59.4	59.0	6.3	6.5	12.6	12.3
p-Anisyl	240	$C_{11}H_{14}O_4N_2$	55.5	55.5	5.9	5.9	11.8	11.9
β-Naphthyl	250	$C_{14}H_{14}O_3N_2$	65.1	64.6	5.4	5.4	10.8	11.1

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3. Liwschitz, Y. and Zilkha, A., 1954, *J. Amer. Chem. Soc.*, **76**, 3698.
4. Larsen, P.O. and Kjaer, A., 1960, *Biochim. Biophys. Acta*, **38**, 148

STUDIES IN ABSORPTION SPECTRA. THE ARYLACRYLIC SYSTEM IN THE INFRARED REGION. PART II*

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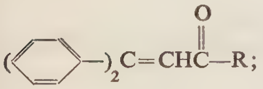
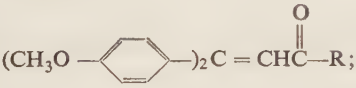
ABSTRACT

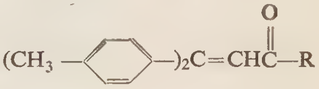
The carbonyl stretching absorption frequencies are correlated for a series of compounds of the types $(Ar)_2C=CHCO_2Ar'$, $(Ar)_2C=CHCONHR$. It is concluded that in the solid state the molecules adopt a coplanar *trans*-cinnamyl conformation with the second aryl group in the β-position twisted out of that plane. The sulfoxyl stretching absorption frequencies of some acids and anilides of the type $(Ar)_2C=CHS(:O)R$, $(R=OH,NHAr)$, are examined too. The C=C absorption frequencies in the 1500–1600 cm^{-1} region are briefly discussed.

The absorption spectra in the IR region (2–15μ) of aryl esters and *N*-arylamides of β,β-diarylacrylic acids and 2,2-diarylethylenesulfinic acids present a very complex pattern of bands. It is the purpose of this paper to discuss the more characteristic bands and to correlate them with the structure of the compounds.

The acrylic esters and acrylamides studied are of the three following types:

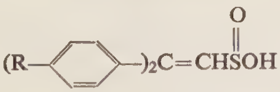


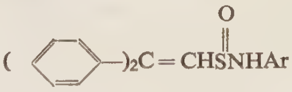




where R stands for OAr, NH_2, or NHAr, and Ar is an unsubstituted or substituted phenyl or naphthyl group.

The 2,2-diarylethylenesulfinic acids and anilides are of the types:





where R stands for various simple substituents.

The compounds studied are much too complicated to draw any definite conclusions on most of their absorption bands, nevertheless, the main source of information regarding the correlations between structure and spectrum of the compounds studied lies in the carbonyl (sulfonyl) stretching absorption frequencies, owing to the large shifts that these frequencies undergo whenever the structure of the vicinity of the carbonyl (sulfonyl) group is changed or there are unsaturated groups conjugated

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* The paper in reference 1 is to be considered as Part I of this series.

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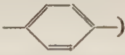
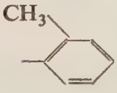
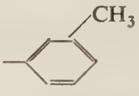
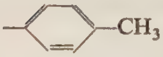
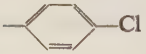
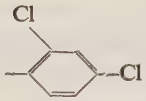
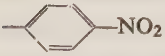
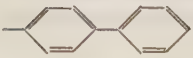
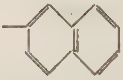
with it. The sulfur-oxygen double bond frequency may have the same privileged situation as the carbonyl frequency and even a better one, because the region in which it appears (about 1090 cm^{-1}) is easy to resolve in spectra obtained from linear wave-length plotting spectrophotometers. Moreover, a further advantage is the absence of strong interfering absorptions arising from other structural features as is the case with the strong carbon–carbon double bond absorption band in the 1600 cm^{-1} region which sometimes is actually superimposed on the carbonyl stretching band. Owing to the scarcity of data on the correlations of the sulfoxyl band and the small number of compounds here inspected, the discussion on this band is not conclusive.

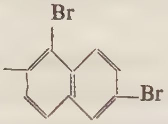
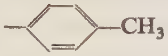
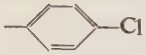
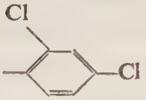
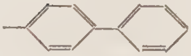
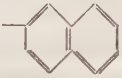
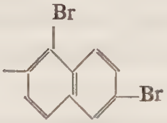
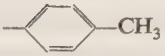
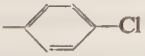
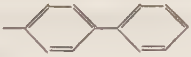
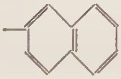
RESULTS AND DISCUSSION

In Tables I–III the frequencies of three characteristic absorption bands of the compounds studied are summarized.

TABLE I

Carbonyl stretching absorption bands and two carbon–carbon double bond absorption bands of aryl esters of the type (X  $)_2C=CHCOOY$

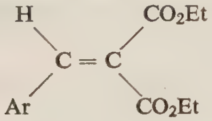
Compound Number	X	Y	C=O $\upsilon(cm^{-1})$	C=C $\upsilon(cm^{-1})$	C=C $\upsilon(cm^{-1})$
I	H	$—C_6H_5$	1718	1590 1609	1484
II	H		1715	1588 1605	1482
III	H		1715	1588 1605	1486
IV	H		1720	1600	1482
V	H		1715	1590 1605	1488 1494
VI	H		1727	1606	1471
VII	H		1733	1593 1614	1489 1520
VIII	H		1706	1572 1591	1480 1509
IX	H		1721	1599 1613	1490 1507

Compound Number	X	Y	C=O ν(cm^{-1})	C=C ν(cm^{-1})	C=C ν(cm^{-1})
X	H		1736	1587 1609	1488
XI	CH_3		1724	1603	1504
XII	CH_3		1706	1601	1484
XIII	CH_3		1733	1603	1475 1507
XIV	CH_3		1724	1598	1484 1509
XV	CH_3		1724	1598	1507
XVI	CH_3		1730	1598	1488 1508
XVII	CH_3O		1698	1598	1501
XVIII	CH_3O		1704	1598	1482 1509
XIX	CH_3O		1706	1582	1500
XX	CH_3O		1718	1588	1484 1505

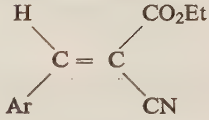
The carbonyl stretching bands of the β,β-diarylacrylic esters studied (Table I) appear in the 1698–1736 cm^{-1} region and those of the amides (Table II) are found in the 1626–1656 cm^{-1} region. These locations correspond approximately to the expected carbonyl frequencies of α,β-unsaturated esters and of primary amides respectively[2]. Nevertheless, it may be useful to compare these carbonyl frequencies with those of similar compounds already known.

Felton and Orr[3] examined the carbonyl frequencies of some aliphatic esters of ethylene-mono- and dicarboxylic acids and cyanoethylenecarboxylic acids in carbon tetrachloride and in chloroform. They found that cross conjugation of a cyano group with the carbonyl group and the steric effect of β-substitution *cis* to the carbonyl group cause a hypsochromic shift relative to the carbonyl absorption bands of the unhindered compounds. Steric strain and electronegative α-substitution were found to have similar effects on various types of saturated and unsaturated esters studied by Rasmussen and Brattain[4].

In part I of this series[1] it was reported that esters of types $ArCH{=}C(CN)CO_2Et$ and $ArCH{=}C(CO_2Et)_2$, where Ar represents variously substituted phenyl groups, have carbonyl stretching absorption bands in the 1707–1734 cm^{-1} and 1724–1747cm^{-1} regions respectively, when determined in the solid state. The bathochromic shift caused by the change of an ethoxycarbonyl group to a cyano group in these series of compounds was attributed mainly to the configuration of the cyanoesters (B, cyano and phenyl groups *cis* to each other) that release most of the steric strain present in the diesters (A). The configurations of both series are as follows:



(A)

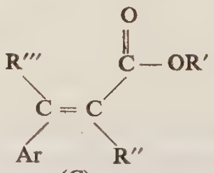


(B)

The less bulky cyano group of (B) permits the aryl group to adopt a conformation that allows the remaining *β*-arylacrylic system to be almost coplanar, and this causes a bathochromic shift with respect to the carbonyl absorption of the diesters (A), in spite of the opposite effect that should be expected from the results for *α*-electronegative substitution mentioned above [2,3].

The carbonyl stretching frequencies of the esters studied in this paper (I–XX) lie in a region of frequencies somewhat lower than those of the diesters (A) and cyanoesters (B) studied in Part I. This may be attributed to the following main characteristics arising from the comparison of the structures of the compounds.

The esters I–XX as well as (A) and (B) have in common the generalized structure (C). It is known that generally aryl esters absorb at higher carbonyl frequencies than the corresponding ethyl esters[4,5].



(C)

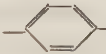
Consequently we have to expect higher carbonyl frequencies when R′ is an aryl group, as in I–XX, than in esters (A) and (B), were it not for the marked influence that may be expected from the groups R″ and R‴. Nevertheless, it may be noted in Table I, that although a small influence can be attributed to the groups X, an increase in the electronegativity of the group Y corresponds roughly to a more noticeable shift of the carbonyl band to a higher frequency; *e.g.*, compounds IX, XV and XX have a varying X group (H, CH_3, CH_3O) and a constant Y group (β-naphthyl), they absorb at 1721, 1724 and 1718 cm^{-1} respectively, on the other hand compounds (with X-group = H) I, V, VI, VII, X, (with Y being C_6H_5, 4-ClC_6H_4, 2,4-$Cl_2C_6H_3$, 4-$NO_2C_6H_4$, 1,6-Br_2-β-$C_{10}H_5$) absorb at 1718, 1715, 1723, 1733 and 1736 cm^{-1} respectively.

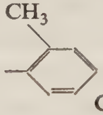
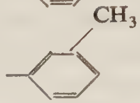
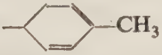
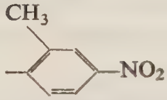
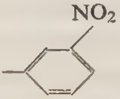
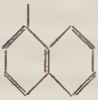
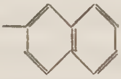
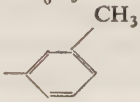
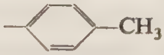
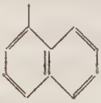
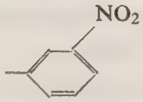
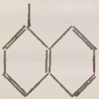
The change of the R″ group in (C) from a cyano group (B) or a carbethoxy group (A), to a hydrogen atom (I–XX) is expected to cause a bathochromic shift of the carbonyl band due to the relief of both conjugative and steric effects. The effect of cancelling the cross-conjugation is to be expected mainly when the cyano group is changed for a hydrogen atom; the former, being more electronegative than the carbonyl group of the ester will tend to conjugate preferentially with the remaining styrenic system, thus depriving the carbonyl group to a considerable extent of conjugation with the unsaturated system. The relief of steric strain caused by the interaction of the aryl group and the α-acrylic substituent *cis* to it in (A) and (B) is accomplished when R″ is a hydrogen atom, as in compounds I–XX, and in this case a coplanar conformation of the partial system Ar—C=C—C=O of (C) may be eventually attained, although some restrictions are then to be imposed on the R‴ group. This coplanar system is attainable only under strained sterical conditions in (A) and (B), as may be seen from molecular models.

The R‴ group in the *cis*-β-acrylic position of (C) is actually capable of disrupting the favourable coplanar conformation described in the preceding paragraph, *i.e.*, R‴ being in compounds I–XX an aryl group it would hinder the carbonyl group in the *cis*-position by adopting a conformation coplanar with the ethylenic double bond. It may be suggested that the R‴ aryl group does not compete successfully against the second aryl group in the β-position and cannot attain coplanarity with the acrylic ester system. This explanation is well supported by several facts: substitution in the aromatic ring in the β-position of the acrylic system has only small effects on the carbonyl absorption frequency, as was seen to be the case of the X group in Table I and in Part I of this series, where a wide range of substituents in the *para*-position of benzylidenemalonic and benzylidenecyanoacetic esters was examined. This is a confirmation of the not too widely studied fact that γ,δ-unsaturation has only a small bathochromic effect on the carbonyl band of an α,β-unsaturated ester. Furthermore, if the R‴ aryl group in (C) would have a strong hindering effect on the *cis*-carboxylic ester group of compounds I–XX, we would have to expect a carbonyl frequency at about the same range as that of the benzylidenemalonic esters studied in Part I, that is 1720–1747 cm^{-1} and not in a range starting as low as 1698 cm^{-1}. The overlap in the higher frequencies (up to 1736 cm^{-1}) may reasonably be supposed to be due to the fact that esters I–XX are aryl esters and not ethyl esters as were those of Part I. (See discussion of R′ in structure (C) above).

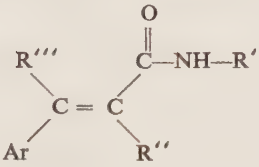
A reasonable conclusion to the above arguments is that the R‴ group does not have a strong hindering effect upon the attainment of coplanarity in the rest of the molecule, or, in other words, the R‴ aryl group is not coplanar with the acrylic double bond. The loss in resonance energy due to this conformation is made up by the gain in resonance stabilization of the second aryl group attached to the β-acrylic position and by the release of the steric hindrance caused by the interaction of R‴ and the ester group.

TABLE II

Carbonyl stretching absorption bands (amide I band) and two carbon–carbon double bond absorption bands of amides of the type (X —*—)$_2$C=CHCONHY*

Compound Number	X	Y	C=O ν(cm⁻¹)	C=C ν(cm⁻¹)	C=C ν(cm⁻¹)
XXI	H		1634	*ca.* 1600	1483
XXII	H		1647	1610	1490
XXIII	H		1650	1603	*ca.* 1500
XXIV	H		1634	1605	*ca.* 1500
XXV	H		1639	1593	1480
XXVI	H		1645	1603	1489
XXVII	H		1645	1592	1497
XXVIII	CH_3	—H	1639	1609	1506
XXIX	CH_3	—CH_3	1645	1620	*ca.* 1500
XXX	CH_3	—C_6H_5	1656	1603	1498
XXXI	CH_3		1647	1603	1489
XXXII	CH_3		1647	1600	*ca.* 1500
XXXIII	CH_3		1656	1600	1492
XXXIV	CH_3O	—H	1626	1598	1503
XXXV	CH_3O		1639	1593	*ca.* 1500
XXXVI	CH_3O		1639	1594	1480

The same arguments may be applied almost without any change, when discussing the carbonyl frequencies of the β,β-diarylacrylamides (XXI–XXXVI), although these arguments are less conclusive owing to the nature of the amidic carbonyl band (amide I band). It is a known fact that prediction of shifts in this band is difficult[2] owing to the marked influence of the orientation of the molecules in the crystalline lattice on the frequency. Nevertheless, in Table II we find the same trend that was discussed above for the case of the esteric carbonyl band, namely, the change of the substituent X has no appreciable effect on the frequency, although a definite but unpredictable influence of the Y group is noted. Furthermore, the range of absorption of this band is 1626–1656 cm^{-1}, considerably less than that found for the derivatives of benzylidenecyanoacetamide (1689–1701 cm^{-1}) and the derivatives of benzylidenemalonamide (1667–1681 cm^{-1}) reported in Part I. Here, again, we have a structure of the type (D) common to compounds XXI–XXXVI and to the amides in Part I.



(D)

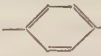
When R′ is an aryl group it generally produces a hypsochromic shift in the amide I band as compared to the corresponding unsubstituted amide [2,6].

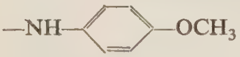
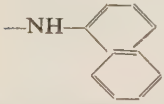
The change of the group R″ from a cyano group to a hydrogen atom is expected to cause a bathochromic shift in the carbonyl band, as was seen in the case of the esters I–XX, owing to the cancelling of cross-conjugation with the carbonyl group and the release of steric interaction with the aryl group in the β-position. On the other hand, the effect of the change of the R″ group from a carbonyl group to a hydrogen atom is unpredictable on the basis of the known facts, the reason being that though cross-conjugation and steric hindrance are cancelled as before, we also eliminate the strong intramolecular hydrogen bonding effect that the carbamyl group is capable of producing. Similarly, lower carbonyl frequencies of benzylidenmalonamides as compared to those of benzylidencyanoacetamides were attributed in Part I to hydrogen bonding, despite the stronger steric hindrance in the former that tends to produce the opposite effect.

The R‴ group would be expected to have the same hindering effects in compounds XXI–XXXVI as those described for compounds I–XX, if it had to attain a coplanar conformation with the acrylic system, with the consequent hypsochromic shifts, allowing, perhaps, for some degree of uncertainty shifts for the amide I band.

Without repeating the arguments which are essentially the same as in the case of the esters discussed above, it is concluded therefore, that the anilides XXI–XXXVI have a conformation similar to that of the esters I–XX.

TABLE III

Sulfoxyl stretching absorption bands and two carbon–carbon double bond absorption bands of compounds of the type (*X* ——$)_2C{=}CHS(:O)Y$

Compound Number	X	Y	S=O v(cm^{-1})	C=C v(cm^{-1})	C=C v(cm^{-1})
XXXVII	H	OH	1059	1580 1600	1500
XXXVIII	CH_3	OH	1029	1603 1627	1521
XXXIX	F	OH	1025 1059	1609	1516
XL	Br	OH	1019 1052	1600	1500
XLI	H	$—NHC_6H_5$	1042 1055	1606	1496
XLII	H		1042	1580 1607	1511
XLIII	H		1038	1606	1496

The sulfoxyl stretching absorption bands in Table III were tentatively assigned on the basis of the results obtained by Detoni and Hadzi[7] for some sulfinic acids; these authors found absorption bands in the 990–1090 cm^{-1} region that were attributed to the sulfoxyl stretching modes, this region corresponding to that of the sulfoxide mean frequency given by Barnard *et al*[8].

The lack of data on this band in the literature does not permit any comparative study, but it seems fair to suppose that the conformation of compounds XXXVII–XLIII is not different from that of their carbonylic counterparts I–XXXVI. It is not unreasonable to suppose, that the sulfoxyl group behaves similarly to the carbonyl group.

The discussion on absorption bands other than those of carbonyl and sulfoxyl is obscured by the complexity of the compounds, for instance, the two carbon–carbon double bond frequencies in Tables I–III can be attributed either to skeletal in-plane vibrations of the rings or the higher frequencies may be eventually ascribed to C=C stretching, for instance of the ethylenic double bond conjugated with both phenyl and carbonyl[2] or sulfoxyl groups; a further obscuring feature is that of the amide II band appearing in the same range of frequencies[2,6]. Nevertheless, it may be mentioned that all the expected bands, correlated with the structural features of the compounds studied appear in their respective spectra.

EXPERIMENTAL

Compounds I–XXXVI were prepared as described in reference 9. Compounds XXXVII–XLIII were prepared as described in reference 10.

Infra-red absorption spectra were determined in the solid state by the potassium bromide pellet technique in a Baird–Atomic Inc. spectrophotometer equipped with sodium chloride optics.

ACKNOWLEDGEMENT

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ABSOLUTE REACTION RATE THEORY—AN ALTERNATIVE DERIVATION

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ABSTRACT

The usual absolute reaction rate expressions for the rate of the reaction A + BC ⟶ AB + C are derived by defining an activated complex with a short constant life time and comparing it with a fictitious complex having a real antisymmetrical valency vibration with a very low frequency.

The rate of the simple chemical reaction

$$A + BC \longrightarrow AB + C$$

is obtained according to the theory of absolute reaction rates by calculating the rate of decomposition of an activated complex ABC*. This complex is assumed to be similar to an ordinary molecule except for the fact that one of its vibrational degrees of freedom is abnormal. The usual approach[1,2] which treats this degree of freedom as a translational degree of freedom along the so-called reaction coordinate, is quite sound but rather difficult to grasp at an elementary level.

A second approach[3,4,5], somewhat easier to comprehend in spite of its lack of rigour, treats this abnormal degree of freedom as a real vibration of very low frequency. The close analogy, and, at the same time, the fundamental difference between this abnormal degree of freedom and a real vibrational degree of freedom are clearly seen when we compare the above chemical reaction with the antisymmetrical valency vibration of an ordinary triatomic molecule DEF. In the first half of each vibration period the interatomic distance DE is decreasing while EF is increasing, whereas in the second half the opposite occurs. The first half of this vibration period is thus very similar to the above chemical reaction in which A approaches B while C recedes from it; however, in the case of the chemical reaction there is no second half to the vibration and C continues to recede from B indefinitely owing to the absence of a restoring force. This is due to the form of the potential energy curve of this degree of freedom, the minimum which would correspond to a real vibration being replaced by a maximum in the case of the chemical reaction[6]. This represents the potential barrier which must be surmounted for chemical reaction to take place; if the atom A and the molecule BC approach each other sufficiently rapidly for the top of this potential barrier to be

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reached, then, on this simple model, chemical reaction will inevitably occur. The existence of a maximum instead of a minimum in the potential energy curve corresponds to a *negative* restoring force and hence to an *imaginary* vibration frequency[5,6]; it is therefore not justifiable to treat this degree of freedom as a real vibration of very low frequency as is done in the second approach mentioned above. The object of the present paper is to obtain the usual absolute reaction rate expressions for the rate of a chemical reaction by modifying this approach so as to free it from the above inconsistency.

We shall find it convenient to employ an activated complex ABC* which differs somewhat from that usually considered; we define this complex as consisting of the group of atoms A, B and C (formed from A and BC and decomposing to give AB and C) from a time $\frac{1}{2}\tau$ before passing the top of the potential barrier till a time $\frac{1}{2}\tau$ after passing the top of this barrier. Our activated complex therefore has a constant life time τ which can be made as short as we please. Our next step is to use equilibrium theory to calculate the concentration of this activated complex and hence to obtain the rate of our chemical reaction given by $[\mathrm{ABC}^*]/\tau$. The calculation can be simplified by considering two fictitious complexes, a "stiff" complex $\overline{\mathrm{ABC}}$ and a "flabby" complex $\mathrm{ABC}^{\sim}$, which resemble triatomic molecules in having real antisymmetrical valency vibrations, but are otherwise identical with the activated complex ABC*. In particular, we suppose that the total energy (including the zero point energy) of the ground state of each of these fictitious complexes is equal to the minimum energy of the activated complex. The "stiff" complex, identical with the "normal molecule with a stiff vibration" considered by Glasstone, Laidler and Eyring[7], is postulated to have a very high frequency for its antisymmetrical valency vibration, so that this vibration is not excited to any appreciable extent. On the other hand, we postulate that the frequency ν of the antisymmetrical valency vibration of the "flabby" complex $\mathrm{ABC}^{\sim}$ is so low ($h\nu << kT$) that this vibration is completely excited and has a practically continuous (classical) energy distribution.

We now consider the rate of the forward reaction $\mathrm{A} + \mathrm{BC} \longrightarrow \mathrm{AB} + \mathrm{C}$ in a fictitious system containing A, BC, AB, C, $\overline{\mathrm{ABC}}$, and $\mathrm{ABC}^{\sim}$ in dynamic equilibrium; this system will contain equilibrium concentrations, not only of A, BC, AB, C, $\overline{\mathrm{ABC}}$, and $\mathrm{ABC}^{\sim}$, but also of the activated complex of the forward reaction ABC*, as well as that of a similar activated complex corresponding to the back reaction. Since the "stiff" and "flabby" complexes differ only in their antisymmetrical valency vibrations, the ratio of their equilibrium concentrations will be equal to the ratio of the corresponding vibrational partition functions, defined in terms of energy levels measured from the corresponding ground states. The vibrational partition function of the "stiff" complex $\overline{\mathrm{ABC}}$ will therefore be unity, while that of the "flabby" complex $\mathrm{ABC}^{\sim}$ will be $kT/h\nu$. It follows that

$$[\mathrm{ABC}^{\sim}] = (kT/h\nu)\,[\overline{\mathrm{ABC}}].$$

The "flabby" complex $\mathrm{ABC}^{\sim}$ will resemble the activated complex ABC* during that part of its antisymmetrical valency vibration in which A is approaching B, C is receding from it and the potential energy is near its minimum value. This resemblance will last from a time $\frac{1}{2}\tau$ before the complex $ABC^{\sim}$ passes through its potential energy minimum to a time $\frac{1}{2}\tau$ after it has passed through this minimum, the only difference between the complexes $ABC^{\sim}$ and ABC^{*} during this time being that one is passing through a potential energy minimum and the other a maximum. By choosing a sufficiently small value for τ this difference will virtually disappear since both these complexes can then be considered as having constant potential energy[a] during the short time interval τ. Finally we note that since the energy of our activated complex ABC^{*}, corresponding to its abnormal vibrational degree of freedom, has its origin in the classically distributed translational energies of A and BC before collision, it will itself have the same classical energy distribution[b] as that of the antisymmetrical valency vibration of the "flabby" complex $ABC^{\sim}$. It follows that the equilibrium concentration of the activated complex ABC^{*} must be the same as the equilibrium concentration of those molecules of the "flabby" complex $ABC^{\sim}$ which resemble it[c], i.e. which have passed through their potential energy minimum in the appropriate direction within the last $\frac{1}{2}\tau$ time interval or will be doing so within the next $\frac{1}{2}\tau$ time interval. Since every molecule of the "flabby" complex $ABC^{\sim}$ resembles the activated complex ABC^{*} for a time τ in every vibration period of duration $1/\nu$, it follows that the equilibrium concentration of the activated complex will be given by:

$$[ABC^{*}] = \{\tau/(1/\nu)\}\ [ABC^{\sim}] = (\tau kT/h)\ [\overline{ABC}].$$

The rate of our chemical reaction v will therefore be given by

$$v = [ABC^{*}]/\tau = (kT/h)\ [\overline{ABC}],$$

the fundamental equation of absolute reaction rate theory.

Since the equilibrium constant $K^{\ddagger}$ of the fictitious equilibrium $A + BC \rightleftharpoons \overline{ABC}$ is given by

$$K^{\ddagger} = \left(\frac{[\overline{ABC}]}{[A]\,[BC]}\right)_{\text{Eq.}} = \frac{Q_{\overline{ABC}}}{Q_{A}\ Q_{BC}}\, e^{-\Delta U_0/RT}$$

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[a] A similar argument is required to justify the usual method of treating the abnormal vibrational degree of freedom of the activated complex as a translational degree of freedom.

[b] It should be noted that our activated complex has a classical energy distribution corresponding to two square terms, whereas the activated complex defined in references (1) and (2) has a classical energy distribution corresponding to only one square term.

[c] This statement, while intuitively reasonable, follows directly from classical statistical mechanics, since both the Hamiltonian function and the volume of phase space corresponding to our activated complex ABC^{*} and to those molecules of the "flabby" complex $ABC^{\sim}$ which resemble it, will become identical as τ tends to zero.

it follows that the above expression for the rate of our chemical reaction can be written in the form

$$v = \frac{kT}{h} K^{\ddagger}[A][BC] = \frac{kT}{h} \frac{Q_{\overline{ABC}}}{Q_A Q_{BC}} e^{-\Delta U_0/RT}[A]\,[BC],$$

the usual absolute reaction rate expressions for the rate of our chemical reaction. In these equations, concentrations are measured in molecules per cc, while the various partition functions Q are calculated for a volume of 1 cc and are defined in terms of energy levels measured from the ground states of the molecules concerned. ΔU_0 is the difference in energy per mole between the ground state of the "stiff" complex $\overline{ABC}$ and that of the ground states of the reactants A and BC. It is therefore also equal to the difference in energy per mole between the minimum energy of the activated complex ABC* and the ground state energy of the reactants A and BC.

It should be noted that since the fictitious complexes $\overline{ABC}$ and $ABC^{\sim}$ take no part in the chemical reaction $A + BC \longrightarrow AB + C$, the expressions obtained above for the reaction rate will still hold exactly for a real system containing A, BC, AB and C in dynamic equilibrium. Finally, just as in the usual derivation of absolute reaction rate theory, the question arises[2] whether the above expressions for the rate of the forward reaction will hold when the reaction products are not present in equilibrium concentrations. Fortunately, except for very fast reactions, this is indeed the case, at least to a good approximation[8].

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PAPER CHROMATOGRAPHY AND PAPER ELECTROPHORESIS OF METHYLPHOSPHONIC, METHYLPHOSPHINIC AND DIMETHYLPHOSPHINIC ACIDS AND OF TRIMETHYLPHOSPHINE OXIDE

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ABSTRACT

A method was developed for separating and determining mixtures of methylphosphonic acid ($MePO_3H_2$), methylphosphinic acid (MeHPOOH), dimethylphosphinic acid (Me_2POOH), trimethylphosphine oxide (Me_3PO), and several inorganic phosphorus oxyacids.

Paper chromatography and electrophoresis techniques have been applied to the separation of inorganic[1,2] and organic derivatives [3,4,5] of the oxyacids of phosphorus. In connection with an investigation on the P^{32}-containing non-volatile products from the neutron activation of trimethylphosphine[7], it was necessary to develop both paper chromatography and electrophoresis techniques for separation and identification of several methyl derivatives of phosphorus.

EXPERIMENTAL

Paper chromatography was carried out by ascending technique for 17 hours using sheets of Whatman No. 1 paper (washed with 2% versene), and a solvent mixture of n-propanol, conc. ammonia and water (6:3:1)[6]. Aliquots of about 0.01 ml of about 0.1 molar solutions of the phosphorus derivatives were used for each analysis. The total length of the solvent front was 28–29 cm in all experiments.

Paper electrophoresis was made[1,8] on $2.5 \times 90 cm^2$ strips of Whatman No. 3 MM chromatography paper, soaked in 0.1 M lactic acid, which were pressed between horizontal glass plates ($15 \times 60 cm^2$ area). The inner surfaces of the plates had been smeared with a thin layer of Silicone grease. Potentials of up to 4000 volts were applied, with cooling of the top plate by tap-water, but best results were obtained with a potential of 750 and about 3 mamp. for three hours without using external cooling. Similar results were obtained with oxalic acid (1%) washed paper strips, or with unwashed paper. This was therefore used for most experiments.

Detection of spots containing phosphorus was made either by an ammonium molybdate spray[5] or by neutron activation[2], using the reaction $P^{31}(n,\gamma)P^{32}$. For neutron activation the paper was dried with warm air, cut in 1 cm strips, wrapped in polyethylene foil and irradiated in a pneumatic tube of the reactor at 2.5×10^{12} n/cm²/sec (pile at 1 Mwatt) for thirty seconds. After about one week (to allow for decay of 15 hours Na^{24}), the P^{32} activity of each 1 cm strip was counted separately with an end-window proportional counter.

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Neutron activation was the only method for detection of trimethylphosphine oxide, which did not give a clear stain with the molybdate spray. The other compounds were detected equally well by both methods. The molybdate spray involves less labour, but the activation method is more sensitive, and also provides a quantitative measure of the distribution of phosphorus in each fraction.

MATERIALS

Methylphosphonic acid, m.p. 103–5° (from benzene) was obtained by hydrolysis with conc. hydrochloric acid of dimethyl methylphosphonate (b.p. 78–9°/18 mm, prepared by refluxing 16.7g of trimethyl phosphite with 8.4 ml of methyl iodide).

Methylphosphinic acid, $CH_3PO_2H_2$ was obtained by hydrolysis of methyldiiodophosphine. Found: Equivalent wt. for Br_2, 303g. CH_5O_2P requires 300g. Details of preparation will be published elsewhere.

Dimethylphosphinic acid, was prepared as described[9], m.p. 89°.

Trimethylphosphine oxide, was obtained by introducing portions of air through a stopcock into a tube containing trimethylphosphine. After 40 hours, absorption of air was found to be complete, and colourless very hygroscopic crystals had formed in the tube [10].

Disodium dihydrogen hypophosphoric acid was prepared as published[11].

RESULTS

R_F values by paper chromatography are given in Table I. Paper electrophoresis results are tabulated as R_P, the ratio of distances of peak maxima to those of the orthophosphate ion. The movement of orthophosphate was 13.9 ± 0.6 cm (at 750 volts during 3 hours). The reproducibility of R_F values in chromatography was much better than that of R_P values in electrophoresis.

TABLE I

Paper chromatography and paper electrophoresis of phosphorus compounds

	R_F Paper Chromatography	R_P Paper Electrophoresis
$Me_2P(O)Me$	0.76 ± 0.05	0.15 ± 0.1
$Me_2P(O)OH$	0.62 ± 0.06	0.37 ± 0.06
$Me(H)P(O)OH$	0.56 ± 0.04	0.50 ± 0.06
$H_2P(O)OH$	0.64 ± 0.1	1.11 ± 0.04
$Me\text{-}P(O)(OH)_2$	0.29 ± 0.06	0.69 ± 0.06
$H\text{-}P(O)(OH)_2$	0.29 ± 0.05	1.28 ± 0.02
K_2HPO_4	0.17 ± 0.02	1
$Na_4P_2O_7$	0.09 ± 0.02	1.34 ± 0.03
$H_2O_3P\text{-}PO_3H_2$	0.08 ± 0.02	1.40 ± 0.05

The results for the R_F values in paper chromatography of ortho- and pyrophosphate are close to those published[5]; 0.17 and 0.08.

DISCUSSION

It is interesting to note (see Table I), that hypophosphorous acid moves in paper chromatography at practically the same rate as methyl- and dimethylphosphinic acids. The substitution of methyl groups for hydrogen atoms on the phosphorus atom of hypophosphorous acid does not seem to affect the partition coefficient of these acids between the moving and the stationary phase. The same effect appears in the pair methylphosphonic acid and phosphorous acid, which move at equal rates. These groups may, however, be separated by paper electrophoresis. The two methods thus complement each other. For separation of methyl- and dimethylphosphinic acids, which move at fairly similar rates by both methods, oxidation of the solution with a slight excess of bromine was used. Methylphosphinic acid was oxidized to methylphosphonic acid: $Me(H)POOH \longrightarrow MePO(OH)_2$, which was readily determined by paper chromatography. Dimethylphosphinic acid is stable to aqueous oxidation[10]. Hypophosphoric acid was identified by boiling in dilute HCl, and detection of phosphorous and phosphoric acids by paper chromatography.

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PHOTOCHEMICAL AND RADIATION CHEMICAL OXIDATION YIELDS OF FERROUS ION IN 4N H_2SO_4

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ABSTRACT

A spectrophotometric, radiation-chemical and photochemical investigation of aerated aqueous solutions of ferrous sulphate in 4 N H_2SO_4 has been carried out. Contrary to previous experimental results [1,2] no evidence for the existence of a complex of ferrous ions with oxygen or of peroxide in which iron has a higher valency, could be obtained.

INTRODUCTION

Proskumin, and coworkers investigated[1] the radiation-chemical oxidation of the ferrous ion under the action of X rays in aqueous 4 N H_2SO_4 solutions containing oxygen. They reported that under these experimental conditions the initial G value for the disappearance of the ferrous ion, $-G(Fe^{2+})$, is very high, being about four times greater than the initial yield for the formation of the ferric ion.
$-G(Fe^{2+}) = 60\text{–}65$; $G(Fe^{3+}) = 15.5$[1]. This effect was interpreted[1] as due to the formation of a peroxide of iron which slowly decomposes with regeneration of the ferrous ion.

It was proposed that these high yields were caused by excitation processes. Recently Orekhov *et al.*[2] interpreted photochemical results in a similar way. They reported a change in the optical absorption of ferrous sulphate solution when oxygen was added. It was reported that under the action of ultraviolet light on 0.01 M $FeSO_4$ solutions the rate of disappearance of the ferrous ion increases in the 0.8–4.0 N H_2SO_4 concentration region. Yet the rate of formation of the ferric ion remained approximately constant. These results were interpreted by assuming the formation of a complex between the ferrous ion and oxygen which under the effect of ultraviolet light in concentrated acid solution gives rise to a tetravalent ferrate complex which cannot be detected by titration with permanganate. These workers assumed that this peroxy compound was destroyed by boiling and that the presence of chloride and ferric ions enhanced the decomposition.

The photochemical curves obtained by these workers[2] did not show the rate decrease due to the inner filter effect of the accumulating ferric ion[3]. These results are in disagreement with a recent photochemical investigation[3]. The radiochemical results are still controversial [4,5,6].

Considering the importance of this problem regarding the basic mechanism of

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excitation by ultraviolet light and ionizing radiation, we reinvestigated the radiation chemical and photo-oxidation of ferrous ion in 4.0 N H_2SO_4, by simultaneous determination of the concentration changes of both Fe^{2+} and Fe^{3+} ions.

EXPERIMENTAL PROCEDURE

Irradiation by γ-rays was carried out using a 5 curie Co^{60} source, using the Fricke dosimeter.

As an *ultraviolet light source*, a Hg low-pressure lamp run at 80mA and 1000 volts was employed. Light intensity was monitored by a Photovolt photomultiplier unit. Light was filtered by 1 cm of 0.1 M NaCl solution in triply distilled water, to remove the 185 mμ line. Actinometry was carried out using a 0.01 N uranyl oxalate actinometer placed in the irradiated cell. The general procedure was similar to that employed in previous work[3].

Irradiation vessels

Irradiations by γ-rays were carried out in pre-irradiated pyrex glass tubes. Ultraviolet irradiations were performed in a fused quartz vessel. The vessels were cleaned with a CrO_3–H_2SO_4 mixture followed by HNO_3 and finally by triply distilled water.

Spectrophotometric measurements

These were carried out with a Beckman DU Spectrophotometer equipped with an IP 28 photo-multiplier. Absorption measurements of oxygen-saturated and evacuated solutions were carried out in Tunberg-type 1 cm quartz cells.

Analysis

Ferric ion was determined as the sulphate at 305 mμ with a Hilger Uvispek spectrophotometer. The extinction coefficient in 0.8 N H_2SO_4 was determined as 2175 liter $mole^{-1}$ cm^{-1} at 24°C in solutions containing less than 0.02 M $FeSO_4$. The temperature coefficient of the absorption at 305 mμ was found to be 0.7% per degree. The extinction coefficient is *p*H dependent, thus after irradiations in 4 N acid the final concentration was adjusted by dilution to 0.8 N. Spectrophotometric determination of the ferrous ion was carried out at 510 mμ by 1,10-phenanthroline[7], using acetate buffer (*p*H∼3.6) and adding NaF at least 150 times the amount of the ferric ion. As the conversion of ferrous ion did not exceed 3%, special precautions had to be adopted. A pair of matched 1 cm cells was used. Care was taken to ensure that the same cell of the matched pair was always used for the blank solution and the same surface of the cells faced the incident light beam. Under these conditions it was found that the optical densities measured were reproducible within 0.002 units for optical densities between 0.100 and 0.900. Each analysis was repeated 4 times and the reproducibility was within 0.003 optical density units. Independent determinations of the ferrous ion concentration were carried out by potentiometric titration, and by visual end-point determination, using N/100 permanganate.

Materials and solution

Triply distilled water was prepared by redistilling distilled water from alkaline permanganate and from phosphoric acid. Ferrous ammonium sulphate, C.P., and Analar sulphuric acid were used without further purification. In the photochemical experiments the initial ferric ion content of solutions containing 0.02 M ferrous ion did not exceed 2×10^{-5} M.

RESULTS AND DISCUSSION

Spectrophotometric measurements

The absorption spectrum of oxygen-saturated 0.038 M $FeSO_4$ solutions in 0.8 N H_2SO_4 in the region 220–300μ showed a small rise in absorption compared with that of an evacuated solution. A re-evacuation of the solution did not change the absorption spectrum. This effect is due to the thermal oxidation of the ferrous ion. The concentration changes of the ferric ion were determined at 305 mμ ($\varepsilon_{Fe^{3+}}$ = 2220 mole liter $^{-1}$ cm $^{-1}$) and at 254 mμ ($\varepsilon_{Fe^{3+}}$ = 2850 mole liter $^{-1}$ cm $^{-1}$). In Table I we present the optical densities D_λ at wave lengths $\lambda = 254$ mμ and $\lambda = 305$ mμ. A simultaneous increase in the optical densities in these two regions is observed. Using the D_{305} values the concentrations of the Fe^{3+} ion were determined. From the data at 254 mμ the concentration changes of the ferric ion were calculated, assuming that the increase in absorption is due only to the formation of the ferric ion. The comparison of these values of Δ Fe^{3+} with those calculated from the optical densities measured at 305 mμ (Table I) indicates that the increase in absorption of oxygen-saturated ferrous solutions observed by Orekhov *et al.*[2] is probably due to thermal oxidation.

TABLE 1

Oxygen effect on the absorption spectrum of 0.038 *M* $FeSO_4$ *solution in* 0.8 *N* H_2SO_4

	D_{254}	D_{305}	10^5 [Fe^{3+}] mole liter^{-1} λ=305mμ	10^5 Δ[Fe^{3+}] mole liter^{-1} λ=254mμ	10^5 Δ[Fe^{3+}] mole liter^{-1} λ=305mμ
Evacuated solution	0.610	0.045	2.07		
1/2 hour after saturation with O_2	0.695	0.100	4.60	3.0	2.5
1 hour ”	0.730	0.135	6.2	1.3	1.6
2 hours ”	0.810	0.200	9.2	2.9	3.0

Radiation chemical experiments

It was reported [1,5] that the nonequivalence between the amount of ferric ion formed and ferrous ion disappearing in the radiation chemical oxidation is observed only at low doses below 3000 roentgen. Our experiments were conducted under similar conditions, the overall doses being 1000–2000 roentgen. As the changes in ferrous ion concentration were determined as a very small difference between the initial and final concentration, special precautions had to be taken. It was found that inaccurate results have been obtained when solutions for irradiation were introducted into undried irradiation vessels. In this case the decrease in the initial ferrous ion concentration due to slight dilution was sufficient to yield changes in the ferrous ion concentration, of the same order of magnitude as reported by Proskurnin *et al.*[1] These results are presented in Table II.

In these experiments the difference between the initial and final Fe^{2+} concentrations is about 1%, while the quantity of Fe^{3+} formed is about 0.2–0.3% of the initial total concentration. Moisture in the irradiation apparatus causes an uncertainty in the total volume of the solution (5 cc for each irradiation) which is of the same order of magnitude (about 1%).

A set of experiments was carried out in a dried irradiation vessel. The results obtained are presented in Table III.

These data indicate that there is equivalence between the amount of the ferrous ion disappearing and ferric ion produced in these experiments. The differences between overall initial and final total concentrations (Table III) are small and within the experimental range of error.

In our experiments we were unable to detect any quantity of ferrous ion inexplicably disappearing on irradiation of aerated 10^{-2} M $FeSO_4$ solutions in 4 N H_2SO_4 at low doses. These radiation chemical experiments show no evidence for the existence of an iron peroxide.

Photochemical experiments

Photo-oxidation of ferrous ion in aerated 4.0 N H_2SO_4 solutions by ultraviolet light at 254 mμ was carried out. The initial Fe^{3+} ion concentration was about 10^{-5} M. Experiments were performed in dried irradiation vessels. These photochemical experiments showed that up to 3% oxidation the equivalence between the disappearance of the ferrous ion and the production of the ferric ion is maintained within the rather wide range of experimental error. The results are presented in Table IV.

The formation of a ferrous oxygen complex was postulated in the thermal oxidation of ferrous ion by molecular oxygen[8]. However, the spectrophotometric data presented in the present work yield no evidence for the existence of this complex. The nature of light absorbing species in aqueous ferrous solutions is not changed by saturation with oxygen, and no specific effect on the photo-oxidation reaction, initiated by direct excitation of the ferrous ion, could be obtained. Similarly we failed to detect any iron peroxide formation when solutions were irradiated with γ-rays. The presant negative results strongly indicate, that in the photochemical and radiation chemical oxidation mechanism of ferrous sulphate solutions in strongly acid medium and in the presence of oxygen, intermediates and products involving iron peroxide do not exist, and that the reactions are consistent with the results obtained previously in less acidic solutions.

TABLE II

Radiation chemical oxidation of aerated ferrous sulphate solutions in 4.0 *N* H_2SO_4 *by* $Co^{60}\gamma$*-rays. Experiments performed in an undried irradiation vessel*

Initial Concentrations				Final Concentrations				
$[Fe^{2+}]$ M	$[Fe^{3+}]$ M	$([Fe^{2+}] + [Fe^{3+}])$ M	t sec	$[Fe^{2+}]$ M	$[Fe^{3+}]$ M	$([Fe^{2+}] + [Fe^{3+}])$ M	$\Delta[Fe^{2+}]$ M	$\Delta[Fe^{+3}]$ M
1.015×10^{-2}	2.12×10^{-5}	1.0171×10^{-2}	30	1.007×10^{-2}	3.50×10^{-5}	1.0105×10^{-2}	8.0×10^{-5}	1.38×10^{-5}
1.015×10^{-2}	2.12×10^{-5}	1.0171×10^{-2}	60	1.002×10^{-2}	4.87×10^{-5}	1.0069×10^{-2}	1.3×10^{-4}	2.75×10^{-5}

TABLE III

Radiation chemical oxidation of aerated ferrous sulphate solutions in 4.0 *N* H_2SO_4 *by* $Co^{60}\gamma$*-rays in dried irradiation vessels*

Dose absorbed roentgen	Irrad. time sec.	Initial Concentration, M			Final Concentration ,M			$100\,\frac{-\Delta[Fe^{2+}]}{[Fe^{2+}]_0}$	$100\,\frac{\Delta[Fe^{2+}] - \Delta[Fe^{3+}]}{[Fe^{2+}]_0}$	
		$[Fe^{2+}]$	$[Fe^{3+}]$	$([Fe^{3+}]+[Fe^{2+}])$	$[Fe_2{}^{+}]$	$[Fe^{+3}]$	$([Fe^{2+}]+[Fe^{3+}])$		Proskurnin *et al.*	present work
1055	30	1.020×10^{-2}	1.79×10^{-5}	1.021×10^{-2}	1.017×10^{-2}	3.44×10^{-5}	1.020×10^{-2}	0.156	0.662	0.135
674	30	1.020	1.79	1.021	1.016	2.85	1.019	0.106	0.423	0.294
1172	30	1.011	2.06	1.013	1.009	3.91	1.012	0.184	0.736	0.016
2168	60	1.011	2.06	1.013	1.006	5.47	1.012	0.340	1.361	0.160
1846	60	1.008	2.52	1.010	1.001	5.42	1.006	0.290	1.158	0.460
1728	60	1.017	2.62	1.019	1.012	5.33	1.017	0.271	1.085	0.279
1933	60	1.014	2.52	1.016	1.012	5.56	1.017	0.303	1.214	0.103
2051	60	1.014	2.52	1.016	1.011	5.74	1.016	0.322	1.287	0.038

TABLE IV

Photo-oxidation of Fe^{2+} *in air saturated* 4.0 *N* H_2SO_4 *at* 20°*C.* $I_0 = 4{,}59 \times 10^{-4}$ *Einstein liter*$^{-1}$ *min.*$^{-1}$ $[Fe^{2+}]_0 = 0.02$ *M*

t min	$10^4 \Delta[Fe^{2+}]$ M	$10^4 \Delta[Fe^{3+}]$, M	Method of Fe^{2+} determination
3	1.75 ± 0.40	2.07 ± 0.06	titration
7	3.7 ± 0.6	4.3 ± 0.1	1.10 phenantroline
10	4.7 ± 0.5	5.0 ± 0.1	titration
14	6.9 ± 1.0	6.0 ± 0.1	1.10 phenantroline

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NEW OBSERVATIONS ON THE THIATION OF HYDROXYPURINES BY PHOSPHORUS PENTASULFIDE*

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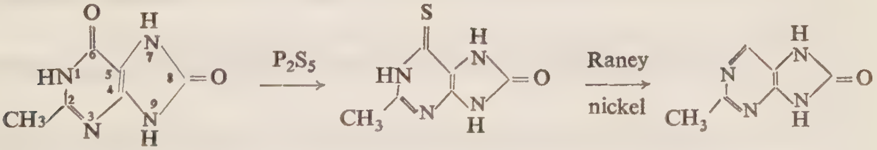
ABSTRACT

6,8-Dihydroxypurine is thiated selectively at position 6. In contrast, 2-methyl-6,8-dihydroxypurine gives as main product the 6,8-dithio derivative, whereas the 6-mercapto derivative is only a minor by-product. It can be shown that 2-methyl-6,8-dithiopurine is not formed from an intermediate 6-, but from the 8-monothio derivative. Therefore, reaction of 2-methyl-6,8-dihydroxypurine with phosphorus pentasulfide follows simultaneously 2 different pathways. The theoretical implications of these observations are discussed.

It was first observed by Beaman[1] that upon interaction with phosphorus pentasulfide, xanthine exchanges only the oxygen atom of the 6-carbonyl group against sulfur. This *selective* substitution has proved of great value in the synthesis of purines which otherwise are difficultly accessible. Surprisingly, this selectivity does not apply to uric acids[2]. The usefulness of the thiation reaction was extended considerably by the recent finding that it is possible to subject 4,5-diaminouracils directly to the action of phosphorus pentasulfide in order to introduce sulfur selectively at position 6[3], and by the additional observation that also in 6,8-dihydroxy-purine attack is confined to the 6-carbonyl group[4].

In an attempt to use the latter reaction for the synthesis of the unknown 2-methyl 8-hydroxypurine (III), the following route was examined, starting with the known 2-methyl-6,8-dihydroxypurine (I)[5].



I II III

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* Dedicated to Prof. M. Frankel, Dept. of Organic Chemistry of the Hebrew University, on the occasion of his 60th birthday, as an expression of friendship and admiration of his scientific achievements.

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When I in pyridine solution was heated with phosphorus pentasulfide for 2 hrs., no reaction took place, whereas the non-methylated homolog of I completes the thiation within 1–1½ hrs.[4] After 4 hrs. reflux a 52% yield of a product was obtained, which was identified as the 6,8-dithio derivative IV by comparison with an authentic sample[5]. From the mother liquor of IV, minute amounts of a new purine, with λ_{max} (pH 8.0) at 242 and 333 mμ, were isolated by paper chromatography. Inspection of the data in Table I suggests that this is the desired 6-thio derivative II. The structure assigned to the by-product is supported by the following observations: a) The 8-thio isomer (V) was synthesised from 2-methyl-6-oxo-4,5-diaminopyrimidine and thiourea. The physical properties of V differed markedly from those of II (Table I).

TABLE I

Physical properties of 6,8-*disubstituted purines and their* 2-*methyl homologs*

Purine	λ_{max} (mμ) at pH			R_F[a]	Fluorescence[b]	Reference
	1.0	8.0	11.0			
6,8-Dihydroxy-purine	255	261	267	0.28	violet	
2-methyl derivative (I)	255	259	273	0.38	violet	A
6-Mercapto-8-hydroxypurine	238 333	237 311	237 310	0.46	ligh blue	B
2-methyl derivative (II)	243 336	242 333	246 314	0.52	violet	
6-Hydroxy-8-mercaptopurine	233 288	235 294	233 289	0.42	violet	B
2-methyl derivative (V)		238 295		0.48	violet	
6,8-Dimercaptopurine	270 357	272 358	241 260 333	0.65[c]	yellow	B
2-methyl derivative (IV)	270 360	273 339	270 335	0.69[c]	yellow	A

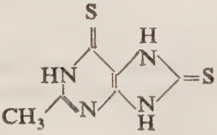
a Determined by the descending method in solvent A (95% ethanol: H_2O: glacial acetic acid = 85: 10: 5, v/v).

b Determined with a Mineralight ultra-violet lamp, emitting light of about 255 mμ.

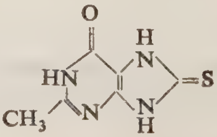
c These R_F values were determined in solvent C (isopropanol): dimethylformamide: 10% NH_3 = 65: 25: 10, v/v).

A see ref. 5.

B Elion, G.B., Goodman, I., Lange, W. and Hitchings, G.H., 1959, *J. Amer. Chem. Soc.*, **81**, 1898.



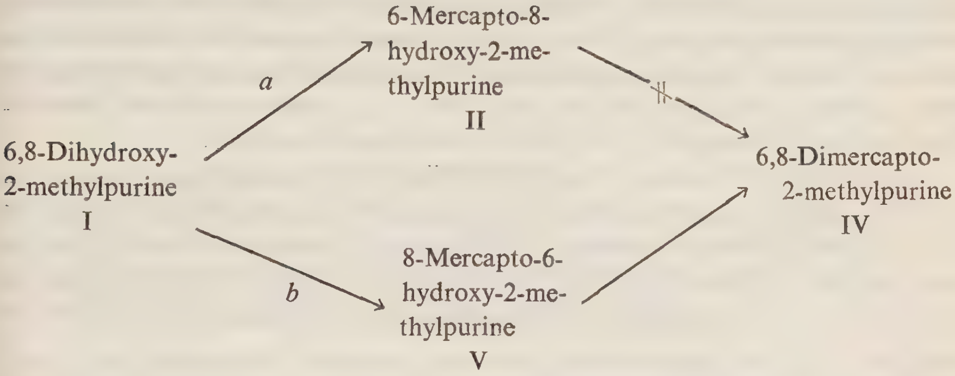
IV



V

b) The absorption maxima of II and their dependence on pH are similar to those of 6-mercapto-8-hydroxypurine, the mother substance of II (see Table I). c) Finally, we succeeded in synthesizing II by an alternative route, which will be described elsewhere. The "by-product" II could thus be identified directly.

Formation of the dithio derivative IV can proceed along two different routes as shown in the following scheme:


6-Mercapto-8-hydroxy-2-methylpurine
II
a
6,8-Dihydroxy-2-methylpurine
I
6,8-Dimercapto-2-methylpurine
IV
b
8-Mercapto-6-hydroxy-2-methylpurine
V

Route *a* would require that in II the 8-carbonyl group becomes susceptible to attack by P_2S_5, in contrast to the behavior of 6-mercapto-8-hydroxypurine, the mother substance of II. This hypothesis would involve the assumption that the second step of thiation proceeds faster than the first, in order to explain our failure to isolate more than traces of II. On the other hand, if route *b* is correct, then 2-methyl-8-mercapto-6-hydroxy purine (V) should react relatively fast with P_2S_5, because not even a trace of the intermediate V was found in the reaction mixture.

Tests carried out with synthetic II showed that this derivative is not attacked by P_2S_5 even after 5 hrs. reflux. On the other hand, the isomer V reacts smoothly with this reagent, conversion to IV being complete within 3–4 hrs. It thus appears that the main route is described by the sequence

$$\mathrm{I} \longrightarrow \mathrm{V} \longrightarrow \mathrm{IV}$$

and that only a very small percentage of I undergoes thiation at C–6, the reaction being stopped at this point.

The change in reactivity produced by the 2-methyl group is remarkable. Two effects are clearly discernible:

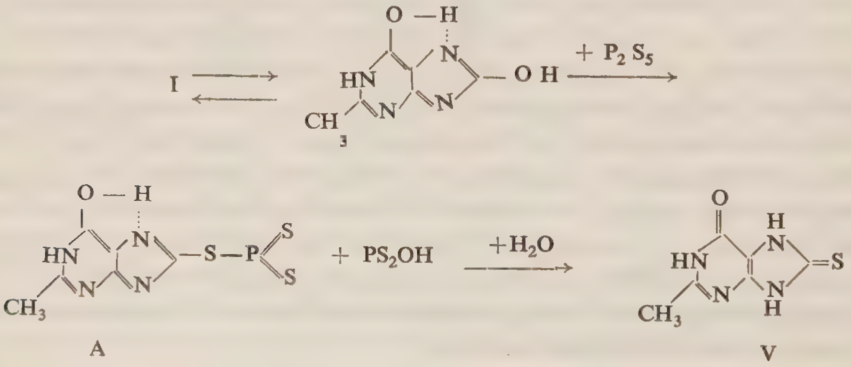
a) De-activation of the 6-carbonyl group;
b) Activation of the 8–carbonyl group.

Thiation by P_2S_5 involves nucleophilic attack at carbon. Therefore, any substituent that reduces the electrophilic character of C-6 in I, will retard the sulfuration reaction at this position. The positive inductive effect of the 2-methyl group, which is transmitted through the conjugated system

$$CH_3-\overset{2}{C}=\overset{3}{N}-\overset{4}{C}=\overset{5}{C}-\overset{6}{C}=O\ ,$$

is responsible for retardation in the case of I. It should be noted that a similar inactivating influence of the 2-methyl group is also evident in the thiation of 6-hydroxy-4,5-diaminopyrimidine. While this compound gives a 50% yield of the corresponding 6-mercaptopyrimidine after 2 hrs. reflux, its 2-methyl derivative was found to be refractory towards phosphorus pentasulfide, as even after 6 hrs. the starting material was recovered unchanged. On the other hand, it is possible to thiate 2-methylhypoxanthine in about 60% yield, albeit under forced conditions[6].

It is more difficult to explain the *activating* effect of the 2-methyl group on thiation of the 8-carbonyl. It should be recalled that neither 6-mercapto-8-hydroxypurine nor its 2-methyl derivative undergoes substitution at position 8. We may therefore conclude that the latter reaction can proceed only if a carbonyl group is present in position 6. A tentative explanation of these findings may be based on the asssumption that thiation at C-8 requires hydrogen bonding between $\overset{6}{C}=O$ and $\overset{7}{N}-H$ and that I reacts in the following "active" form:



In this representation of the thiation of I, the primary product is not the 8-thiopurine V itself, but the thiophosphate ester A, which is decomposed slowly by subsequent heating with water. It is also apparent that the tautomeric shift shown cannot be induced by a 6-mercapto group, in view of its weak hydrogen bonding power Therefore, thiation at C-8 cannot take place in purines bearing a 6-mercapto substituent.

It should also be recalled that P_2S_5 does not attack the 2-carbonyl group of xanthine or uric acid although this position is neighbored by two NH-groups, similar to the arrangement at C-8 in I. This difference indicates that a special intramolecular mechanism must be operative to make the 8-carbonyl susceptible to thiation. Thus it appears probable that — under the influence of the reagent — enolisation can proceed only if certain structural requirements are fulfilled.

These considerations may also shed light on the observation that uric acid gives 2 products when reacting with P_2S_5, viz. 6-thiouric acid and 6,8-dithiouric acid[2]. It appears probable that in this case too the dithio derivative is the result of a second reaction, paralleling route *b* in the above scheme and involving thiation at C-8 as a first step. We assume that 6-thiouric acid is resistant to further attack by the reagent and therefore not an intermediate in the formation of the dithiouric acid. This problem is now under investigation.

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azobenzene and the azonaphthalenes[7] the question arose if isomerism exists in the quinone-phenyl-hydrazones which are tautomeric with the hydroxy-azocompounds, e.g. (VI) ⇆ (VII)

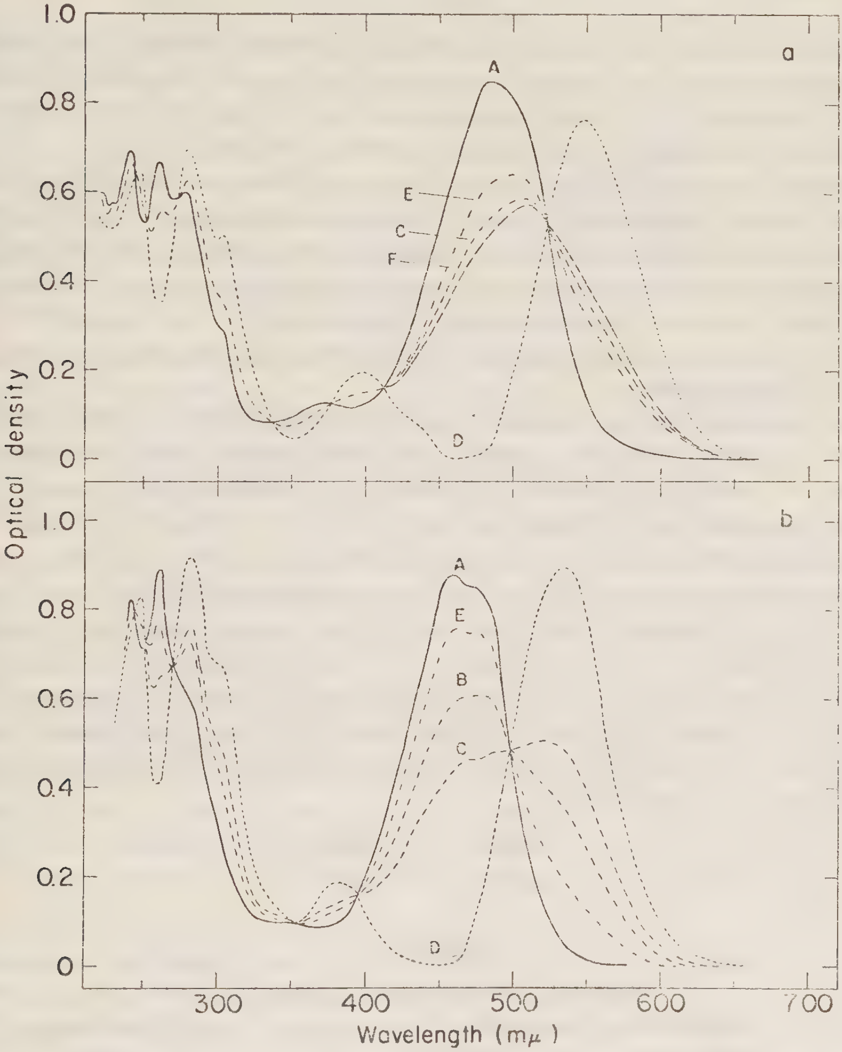
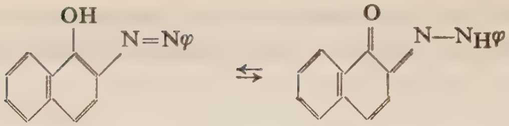
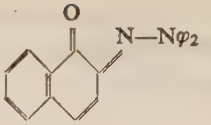


FIGURE 1

Absorption spectra of compound (VIII) at–100° (a) in ethanol, 3.06 × 10^{-4} mole/lit., 1 mm cell; (b) in m.c.h./i.h., 1.53 × 10^{-5} mole/lit., 22 mm cell. Curves A and D are computed for pure "A" and "B" as explained in the text. In ethanol curve A is also practically identical with that of the thermal equilibrium mixture at room temperature, and with the photo-equilibrium mixture at 578 mμ. The other curves are as follows: (a): C—photo-equilibrated at 436 mμ, E—ditto at 510 mμ, F—ditto at 480 mμ. (b): B—photo-equilibrated at 365 mμ, C—ditto at 436 mμ, E—thermal equilibrium mixture at 20°, cooled to–100°.



(VI) (VII)



(VIII)

We therefore started our investigation of photo-isomerisation and of the kinetics of thermal isomerisation in quinoid systems with a study of 1,2-naphthoquinone-2-diphenylhydrazone (VIII), the absorption spectrum of which has been described by Burawoy[8]. The results are reported in the present communication.

When solutions of (VIII) in either polar or non-polar solvents are irradiated with light of different wavelengths, the absorption spectra change markedly, with a well-defined endpoint attained at each wavelength of photo-active light. At room temperature, these irradiated solutions slowly revert to their original state. The reversion is enhanced at elevated temperatures and slowed down at low temperatures. It is a first-order reaction, and the temperature dependence of its rate can be expressed by an Arrhenius equation.

The system thus behaves as a mixture of two compounds "A" and "B", characterized by different absorption spectra, and interconvertible either spontaneously (whereby a thermal equilibrium is established), or photochemically (provided the thermal interconversion is slowed down or prevented by cooling to sufficiently low temperatures). The thermal equilibrium mixture in non-polar solvents at room temperature contains about 84% of "A", as against about 98% in ethanol.

The rates of thermal equilibration in non-polar solvents can therefore be measured in both directions, i.e. A ⟶ B and B ⟶ A. Application of the Arrhenius equation to the results of measurements of these rates at several temperatures gives values for the energies of activation E_A. The enthalpy difference $\triangle H$ between the two isomers can be calculated from the temperature dependence of the thermal equilibrium constant K, according to

$$\frac{d(\ln K)}{d(1/T)} = -\frac{\triangle H}{R}$$

RESULTS

Thermal and photochemical equilibria

The first indication that solutions of (VIII) actually contain two isomers was obtained as follows. A solution of (VIII) in a mixture of methylcyclohexane and isohexane, after being kept in the dark for several days and thus at thermal equilibrium, was cooled to minus 110° and irradiated to saturation with light of various wavelengths. As a result the absorption spectra changed as shown in Figure 1b, where the main band in the visible region, at about 460 mμ, is seen to increase for some wavelengths and decrease for others, while simultaneously the absorption at longer wavelengths falls or rises, due to changes in an absorption band at 530 mμ.

The well-defined isosbestic points throughout the visible and ultraviolet regions provide conclusive evidence for the existence of two interconvertible modifications, which will be identified henceforth by their main long-wave absorption bands; the form absorbing at about 460 mμ will be denoted by "A" and the one characterized by the 530 mμ band, by "B". In order to estimate the absorption spectrum of pure "B" without actually isolating this modification, it will be assumed arbitrarily that "B" does not absorb at 450mμ. This procedure is analogous to the one successfully applied to the computation of the absorption spectra of *cis* isomers of azobenzene and azonaphthalene derivatives[2,7,9], and of azomethines[3], and results in curve D. With regard to pure "A" it is assumed, again arbitrarily, that it does not absorb at 550 mμ. This is virtually the case after irradiation at 546 mμ (Curve A). Similar results were obtained with a solution in ethanol, as shown in Figure 1a. The numerical data of the absorption spectra in both solvents are summarized in Table I.

TABLE I

*Absorption maxima and isosbestic points of solutions of (VIII) in ethanol (a) and in methylcyclohexane-isohexane (b). Wavelengths in mμ, $10^{-3} \times \varepsilon$ values in parentheses, calculated for the pure (A) and (B) modifications whose spectra are shown in Figure 1, curves A and D. *denotes inflexion points.*

Isomer "A"	(a)	510*; 485 (27.8); 370*; 305*; 278 (19.6) 261 (21.5); 240 (22.5)
	(b)	475 + 460 (26.7); — — 280* — 260 (26.7); 240 (24.3
Isomer "B"	(a)	547 (24.8); 400 (6.1); 305*; 280 (22.9); 246 (21.2)
	(b)	530 (26.7); 382 (5.6); 305*; 280 (27.3); 248 (24.6)
Isosbestic points	(a)	523 (17.3); 412 (5.1); 377(3.9);338(2.5); 274(19.3);251(17.3);244(20.9)
	(b)	497 (14.2); 395 (4.7); 355 (3.0); 270(19.6);251(21.7);243(23.4)

The absorption bands of both isomers in ethanol in the visible region are seen to be shifted by about 20 mμ towards longer wavelengths, as compared with solutions in non-polar solvents. The same assumptions, i.e. that absorption at 450 mμ is due only to "A" and at 550 mμ only to "B" (in non-polar solvents), also make it possible to estimate the percentage of the two forms in thermal equilibrium at and above room temperature, and in photochemical equilibrium after photo-equilibration with light of various wavelengths, as shown in Figure 1. The results of such calculations of the composition of the equilibrium mixtures are summarized in Table II.

TABLE II

Photo-equilibria (i.e. isomeric composition after irradiation until no further change is observable) attained at various wavelengths of photo-active light, at–100° (*cf. Figure* 1).

Wavelength mμ	Percent "A" in ethanol	Percent "A" in m.c.h./i.h.
578	100	—
546	—	98
510	72	—
480	59	—
436	63	50
365	—	67
300 + 313	70	—
thermal equilibrium	98	84

Both thermal and photochemical equilibria are little affected by changes in concentration in the range 1.5×10^{-5} to 3×10^{-4} mole/lit. Beer's law was shown to be followed by solutions in methylcyclohexane at room temperature in the range $6 \times 10^{-6} - 3 \times 10^{-4}$ mole/lit. The temperature dependence of the thermal equilibrium can be determined by measuring the absorption spectra at various temperatures in a temperature region where the thermal interconversion is known to be rapid. As will be shown in the second paragraph, this is the case for temperatures above about 20°. The ratio of optical densities at 450 mμ ("A") and 550 mμ ("B") is a measure of the equilibrium constant K = ("A")/("B"), and the temperature dependence of this ratio may therefore serve for the computation of dK/dT. However, since the absorption spectrum of a pure substance is also affected by changes in temperature, the results were checked as follows: Solutions were equilibrated at 20° by keeping them in the dark for a week, quenched by cooling with dry ice, and then measured at 0°. The procedure was repeated following equilibration at 95°. These experiments were carried out with 3.10^{-4} mole/lit. solutions in methylcyclohexane in 1mm cells, to facilitate quenching. The actual absorption spectra characterizing the equilibria at 25° and at 95° are thus both measured at 0°. Both methods show that K is little affected by changes in temperature. The results

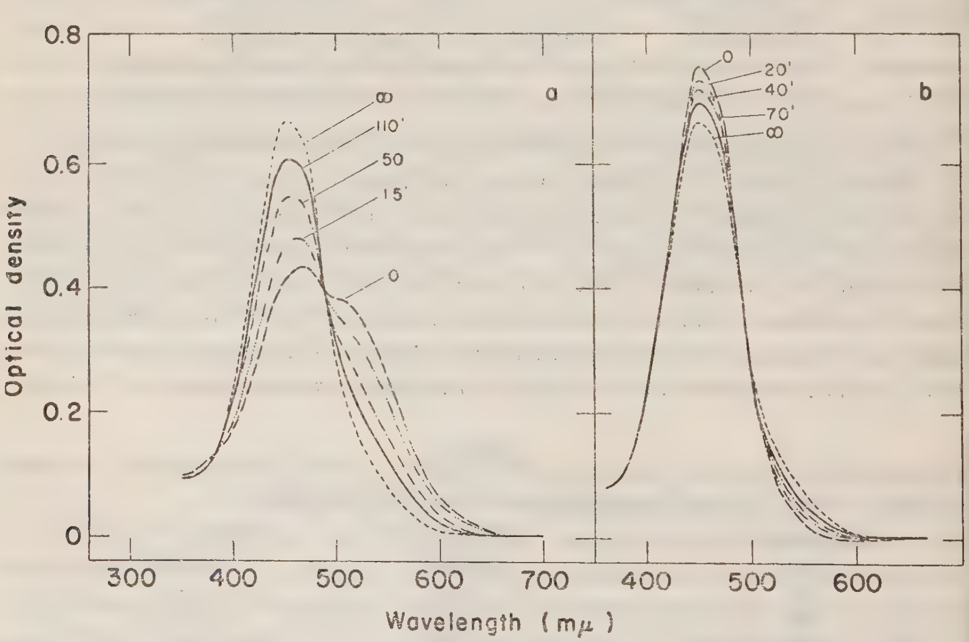


FIGURE 2

Kinetics of the thermal transformation "B" ⟶ "A" at 19° (in Figure 2a) and "A" ⟶ "B" at 16° (in Figure 2b). Absorption curves denoted ∞ are those of the thermal equilibrium mixture. Solutions were measured, irradiated at either 436 mμ (a) or 546 mμ (b) at the above temperatures, and their spectra then taken at the intervals indicated in the Figure (in minutes). The half-times $\tau_{\frac{1}{2}}$ calculated for the two reactions from this Figure are 47′ for "B" ⟶ "A" and 60′ for "A" ⟶ "B".

of a typical experiment were $K_{95^\circ} = 5.0$, $K_{20^\circ} = 5.7$, yielding an enthalpy difference $\triangle H$, between "A" and "B", of about 400 cal./mole.

Kinetics of thermal interconversions

As mentioned in the introduction, the solution of (VIII) in a non-polar solvent contains an equilibrium mixture of the two isomers. This equilibrium can be temporarily disturbed, i.e. moved either in favour of "A" or of "B", by suitable irradiation, which will be followed by a spontaneous return to the thermal equilibrium. A typical experiment is shown in Figure 2. The results of similar experiments in a wide range of temperatures are summarized in Table III, which also includes data for solutions in ethanol at two concentrations. The constants in the Arrhenius equations $\ln k = \ln A - E_A/RT$ calculated from the data of Table III are as follows:

		lg A	E_A(kcal./mole)
"A" ⟶ "B"	in m.c.h., 1.5×10^{-4} m/l	4	10
"B" ⟶ "A"	” ” , ditto		
"B" ⟶ "A"	” ethanol, 3×10^{-4}m/l	8.4	15.6
"B" ⟶ "A"	” ” 3×10^{-5}m/l	9.4	17

The rate measurements in m.c.h. refer of course to $k' = k_1 + k_2$ in $A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B$. The resulting value for E' is defined by $\ln k' = \ln A' - E'/RT$, and is related to E_1 and E_2 (defined by $\ln k_1 = \ln A_1 - E_1/RT$; $\ln k_2 = \ln A_2 - E_2/RT$) by the equation[9a]

$$E' = (k_1 + k_2)^{-1}(k_1E_1 + k_2E_2)$$

Since $\triangle H = (E_2 - E_1)$ has been shown to be close to zero, the value thus calculated for E' may be taken as approximately equal to E_1 and to E_2.

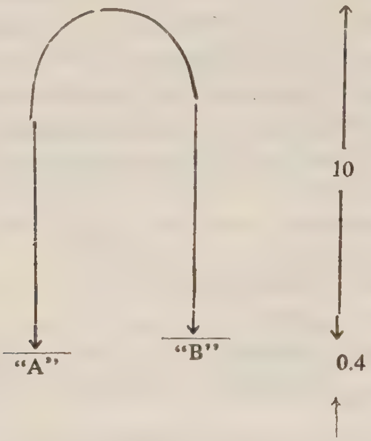
TABLE III

Half-life times $\tau_{\frac{1}{2}}$ (in minutes) for the thermal equilibrations "A" ⇌ "B" at various temperatures, as explained in Figure 2 and in the text.

	in m.c.h./i.h., 1.5×10^{-5} m/l		In ethanol 3×10^{-4} m/l	In ethanol 3×10^{-5} m/l
t, °C	"B" ⟶ "A"	"A" ⟶ "B"	"B ⟶ "A"	"B" ⟶ "A"
5	112	127	102	—
10	—	—	—	74.5
16	—	60	—	—
17.5	—	—	26	—
19	47	—	—	—
25	—	—	—	14
30	25	30	—	—
35	—	—	6.4	—
40	17.5	20	—	—
45	—	—	—	2.4
50	8.5	9	1.8	—
55	—	—	—	1.1

DISCUSSION

The picture emerging from the above results is that compound (VIII) is capable of existing in two interconvertible modifications, "A" and "B", of approximately equal enthalpy, separated by a potential barrier (in non-polar solvents) of about 10kcal./mole, as indicated in the following energy scheme:



The frequency factors of 10^4 found for the thermal isomerisation are extremely small for a first-order reaction. It is because of this that the thermal interconversion of "A" and "B" is so slow even at room temperature, despite the small activation energy.

The photo-isomerisation reactions observed are closely similar to those reported for the *cis* ⇆ *trans* photo-isomerisations of arylazo compounds and azomethine derivatives mentioned in the introduction. However, the present case differs in that the two modifications are of similar energy content, and that in non-polar solvents the equilibrium constant ("A")/("B") is not far from unity. The very pronounced effect of ethanol as solvent, both in shifting the thermal equilibrium almost completely towards "A" and in increasing the activation energy for the process "B"→"A" may be due to the differences in hydrogen bonding between solutes "A" or "B" and the solvent.

From the kinetic data it appears feasible to separate the isomers chromatographically at reduced temperatures, provided that spontaneous interconversion is not promoted by the chromatographic medium (cf. the azonaphthalenes[10]). The present results do not suffice to draw conclusions as to whether or not the "A" and "B" isomers described above are indeed geometrical isomers involving the $C=N$ double bond, although by analogy with arylazocompounds and azomethines such an isomerism appears to be the most plausible one. It is hoped that experiments with the diphenylhydrazone of 1,4-naphthoquinone and with related compounds will clarify the situation. Unfortunately there is little prospect for conclusive evidence from measurements of electric dipole moments, since both isomers should have fairly large moments.

EXPERIMENTAL

Materials

Compound (VIII) was prepared according to Pogany[11], as cited by Burawoy[8], and had a melting point of 173°.

The 1:1 mixture of methylcyclohexane and isohexane serving as a non-polar solvent for use at low temperatures was distilled into the measuring cell in a high vacuum from a potassium/sodium alloy in order to exclude hydroxylic contaminants and air, and the cell then fused off[12]. The two hydrocarbons were first purified by passage through a column of highly active alumina[13]. Ethanol (abs.) was freshly distilled from KOH.

Photochemical and spectrophotometric techniques

These were as described[12]. A Cary Mod. 14 recording spectrophotometer was used. The light source was a Philips Spectral Lamp mercury or mercury/zinc/cadmium arc. The various emission lines were isolated by the usual Corning filter combinations (578, 546, 436, 365 mμ) or by interference filters, (510, 480, 405, 300 + 313 mμ). Photo-equilibrations took between 15 and 80 minutes, depending on the wavelength.

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THE REACTION OF EPOXIDES WITH AMINO ACIDS HAVING SULFHYDRYL GROUPS

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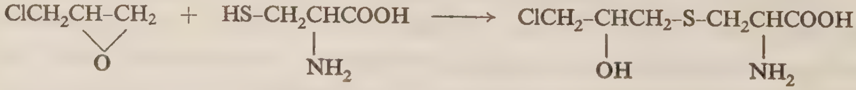
The reaction of epoxides with various functional groups such as hydroxyl, amino and sulfhydryl are well known. The epoxide ring is opened leading to substitution by hydroxyalkyl groups. With amino acids, N-hydroxyalkyl derivatives were formed from which by cyclization 2-morpholones were obtained [1,2]. The reaction of ethylene oxide with cysteine leading to the formation of S-(2-hydroxyethyl)-cysteine has been mentioned[3].

We have investigated the reaction of various epoxides, such as propylene oxide and epichlorhydrin, with amino acids having sulfhydryl groups, such as cysteine in order to prepare S- and N- hydroxyalkyl derivatives of the latter. These derivatives are potential antimetabolites[4,5,6].

Some S-alkyl derivatives of cysteine have been found in nature. Stoll and Seebeck[7] isolated S-allyl-L-cysteine sulfoxide (alliin) from garlic. S-Methyl-L-cysteine sulfoxide has been found in various plants, such as onions[8]. S-Ethyl cysteine has antituberculosis activity[4]. S-Dichlorovinyl cysteine produces fatal aplastic anemia in young calves[5]. N-Hydroxymethyl-methionine exhibits bactericidal and bacteriostatic action[6].

We found that the reaction of one equivalent of epoxide with cysteine in the presence of, e.g., triethylamine led to the formation of S-hydroxyalkyl derivatives exclusively, while the use of two equivalents led also to hydroxyalkylation of the less reactive amino group. Thus one equivalent of propylene oxide gave with cysteine S-(2-hydroxypropyl)-cysteine and two equivalents gave S,N-di-(2-hydroxypropyl) -cysteine. The proposed structure of these derivatives is based on analogy with the reaction of propylene oxide with glycine[2].

The reaction of one equivalent of epichlorhydrin (I) with cysteine gave S-(3-chloro-2-hydroxypropyl)-cysteine (II) showing that the epoxide group is more reactive than the chlorine atom. The opening of the



$$\underset{\text{I}}{\text{ClCH}_2\text{CH--CH}_2 \ (\text{O})} + \text{HS--CH}_2\underset{\text{NH}_2}{\text{CH}}\text{COOH} \longrightarrow \underset{\text{II}}{\text{ClCH}_2\text{--}\underset{\text{OH}}{\text{CH}}\text{CH}_2\text{--S--CH}_2\underset{\text{NH}_2}{\text{CH}}\text{COOH}}$$

I

II

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epoxide ring is probably as given as this is analogous to the reaction of phenyl lithium with epichlorhydrin[9], or to that of the phenoxide ion with epichlorhydrin in the formation of epoxy resins[10].

EXPERIMENTAL

M.p.s. were determined in a Fisher-Jones apparatus. The ascending method of paper chromatography (80% phenol) was used.

S-(2-Hydroxypropyl)-L-cysteine. At 0°, L-cysteine hydrochloride (1.57g, 0.01 mole) was dissolved, under nitrogen, in water (6 ml) and ethanol (6 ml) and propylene oxide (0.6g, 0.01 mole) added, followed by triethylamine (2g, 0.02 mole). The reaction mixture was left in the cold for 30 min. and then kept overnight at room temperature. The reaction mixture gave a negative test for free cysteine with nitroprusside and was evaporated to complete dryness *in vacuo* from a water bath at 50°. The residue was extracted by boiling absolute ethanol to remove triethylamine hydrochloride, and the insoluble substance filtered and washed with absolute ethanol. It was recrystallized from a mixture of a small volume of water, in which it is very soluble, and a large volume of acetone; m.p. 208°; yield, 1g (56%). The substance gave positive ninhydrin reaction; Rf 0.77.

Anal. Calcd. for $C_6H_{13}NO_3S$: C,40.2; H,7.3; N(Kjeldahl),7.8; N(Van Slyke), 7.8. Found: C, 39.8; H,7.2; N(Kjeldahl), 7.8; N(Van Slyke), 7.7.

S,N-Di-(2-hydroxypropyl)-L-cysteine. L-cysteine hydrochloride (1.57g; 0.01 mole) was dissolved, as above, in water–ethanol, cooled in ice water, and propylene oxide (1.3g; 0.022 mole) added followed by triethylamine (2g; 0.02 mole). The reaction mixture was worked up as above. Thus, 1.1g of the disubstitution product were obtained. As the substance is slightly soluble in hot absolute ethanol, concentration of the ethanol extract gave a second crop of 0.3g, free of chlorine; total yield, 1.4g (60%). The compound was recrystallized by addition of a large volume of acetone to a solution in a small volume of water; it had m.p. 214° and gave a negative ninhydrin reaction. On paper chromatograms it gave a weak positive reaction; Rf 1.0.

Anal. Calcd. for $C_9H_{19}NO_4S$: C,45.6; H,8.0 N(Kjeldahl), 5.9; N(Van Slyke), 0.0. Found: C,44.9; H,8.0; N(Kjeldahl), 5.9; N(Van Slyke), 0.0.

S-(3-Chloro-2-hydroxypropyl)-L-cysteine. At 0°, L-cysteine hydrochloride (8g, 0.051 mole) was dissolved, under nitrogen, in water (50 ml) and ethanol (100 ml), and epichlorhydrin (4.6g; 0.05 mole) added, followed by triethylamine (10.1g; 0.1 mole). The reaction mixture was worked up as before. It was evaporated *in vacuo* to complete dryness at 40° and the triethylamine hydrochloride extracted from the residue several times with hot absolute ethanol, until the extract gave a negative test for chloride ion. The residue was dissolved in a minimum quantity of water at 40° and a large volume of acetone added. The substance was left in the cold to crystallize; yield, 3.2g (30%); m.p. 194°. Further recrystallization from water-acetone did not raise the melting point. The substance gave a positive ninhydrin reaction; Rf 0.74.

When pyridine was used instead of triethylamine, the reaction mixture still gave a positive reaction for free cysteine after 24 hrs.

Anal. Calcd. for $C_6H_{12}ClNO_3S$: C,33.8; H,5.6; N(Kjeldahl), 6.6; N(Van Slyke), 6.6. Found: C,34.2; H,5.7; N(Kjeldahl), 6.6; N(Van Slyke), 6.5.

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CARBONYL-METHYLENE CONDENSATIONS PART XIV. DIRECT SPECTROPHOTOMETRIC EVIDENCE FOR THE STEREOCHEMISTRY OF THE UNCATALYSED REACTION

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ABSTRACT

The non-catalysed condensation of *o*- and *p*-methoxybenzaldehyde with ethyl cyanoacetate was studied spectrophotometrically. The results show that the ethyl *trans*-arylidenecyanoacetate (*i.e.*, with the aryl and carbethoxy groups in the *trans*-position) is formed exclusively in the reaction.

In base-catalysed or uncatalysed Knoevenagel reactions usually only one geometrical isomer is obtained, and only a few cases are known in which both *cis* and *trans* products are formed. Bruylants[1] found two isomers in the reaction of *p*-dimethylaminobenzaldehyde with cyanoacetic acid and n the reaction between 3,5-dimethyl-4-nitrobenzyl cyanide and benzaldehyde. Stefanovic[2] found an unstable isomer accompanying the stable ethyl α-nitro-β-(*p*-dimethylaminophenyl)-acrylate in the reaction of *p*-dimethylaminobenzaldehyde with ethyl nitroacetate. Schienez[3] separated two acids from the reaction of 3,4,5-trimethoxybenzaldehyde with cyanoacetic acid and assigned their configurations according to their ease of decarboxylation. Pratt[4] suggested the formation of both isomers of the product in the reaction of benzaldehyde with ethyl acetoacetate, as he did not succeed in obtaining a crystalline product from the reaction mixture although a solid isomer was known previously. Harfenist and Phillips[5] suggested the occurrence of a mixture of *cis* and *trans* isomers to explain the results of the decarboxylation of the condensates of aromatic aldehydes with cyanoacetic acid. Two investigations were carried out determining the stereochemistry of the reaction. Baker[6] synthetised two pairs of arylidenecyanoacetic acids: The *cis* configuration of aryl and carboxyl groups was obtained by opening a coumarin ring, while the normal products formed in carbonyl-methylene condensations were the *trans* isomers. Recently, Zabicky[7] prepared a number of condensation products in kinetic conditions and found no difference between crystals which separated at the very beginning of the reaction or in the later stages, and he concluded from the infra-red spectra that these products have the *trans* configuration. However, both these methods are based on the isolated reaction products, and it was shown by us[8] that ethyl *cis*-*o*-methoxybenzylidenecyanoacetate

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is rapidly isomerised to the *trans* isomer by bases as well as by ethyl cyanoacetate and even by aqueous ethanol. Thus, it is possible that the initially formed isomer could be the *cis* compound e.g., if its formation is favoured kinetically) while its rapid isomerisation makes possible only the separation of the thermodynamically more stable *trans* isomer. Situations like this were described by Zimmerman[9] in the ketonization of enols.

In the case of ethyl *o*-methoxybenzylidenecyanoacetate, both isomers have been obtained[8] by esterification of the corresponding acids[6]. Kinetic spectrophotometric measurements of the reaction between *o*–methoxybenzaldehyde and ethyl cyanoacetate were carried out at four wave-lengths simultaneously, including the wave lengths corresponding to the absorption maximum of the *cis* and *trans* isomers of the product. The corresponding absorption data at these wave lengths both for the pure *trans* and the pure *cis* isomers, as well as the data obtained at various times are given in Table I. As the appearance of any amount of the ethyl *cis*-*o*-methoxybenzylidenecyanoacetate isomer in the solution must introduce changes in the relative absorption values R_1, R_2 and R_4 when R_3 was defined as unity, the close similarity of the R values measured shows that the product of the condensation is exclusively the *trans* isomer. The absorption data obtained at very short reaction times were not used, as control experiments showed that some interfering side reaction took place between the aldehyde and the solvent at the start of the reaction. Nevertheless the values given in Table I are taken from only 0.3% to 1.8% reaction, so that the data still certainly correspond to initial rates.

Although only one isomer is known of ethyl *p*-methoxybenzylidenecyanoacetate, its rate of formation was measured as described above at four wave lengths, and it was found that again only the *trans*-isomer which is formed in the usual synthesis[7] was obtained.

TABLE I

Spectrophotometric measurements of the formation of ethyl o-methoxybenzylidenecyanoacetate from* 0.04 *M o-methoxybenzaldehyde and* 0.18 *M ethyl cyanoacetate at* 40° *in* 95% *ethanol. Values of* R_1, R_2 *and* R_4 *are relative optical densities, taking the optical density(D) at* λ =360 *mμ as unity.*

Time (min.)	107	139	161	195	264	316	363	R_{trans}	R_{cis}
D at 346 mμ	0.128	0.195	0.240	0.302	0.440	0.532	0.635		
$R_1=D_{346}/D_{360}$	0.80	0.82	0.80	0.83	0.84	0.83	0.82	0.81	1.16
D at 349 mμ	0.148	0.218	0.260	0.336	0.478	0.575	0.672		
$R_2=D_{349}/D_{360}$	0.92	0.90	0.92	0.92	0.91	0.90	0.88	0.90	1.15
D at 360 mμ	0.160	0.239	0.282	0.365	0.525	0.640	0.776		
$R_3=D_{360}/D_{360}$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
D at 370 mμ	0.140	0.211	0.250	0.330	0.466	0.590	0.692		
$R_4=D_{370}/D_{360}$	0.88	0.89	0.89	0.90	0.89	0.92	0.90	0.89	0.74

* The molar extinction coefficient ε of the pure *trans*-isomer is 8920, 9420, 10500, and 9400 at 346, 349, 360 and 370 mμ, respectively. The ε values for the *cis*-isomer at the same wave-lengths are 8170, 8110, 7020 and 5400.

TABLE II

Spectrophotometric measurements of the formation of ethyl p-methoxybenzylidenecyanoacetate from* 0.004 *M anisaldehyde and* 0.16 *M ethyl cyanoacetate at* 40° *in* 95% *ethanol. Values of* R_1, R_3 *and* R_4 *are relative optical densities, taking the optical density (D) at* λ =344 *mμ as unity.*

Time (min.)	58	80	94	156	220	R_{trans}
D at 332 mμ	0.073	0.123	0.155	0.275	0.410	
$R_1 = D_{332}/D_{344}$	0.88	0.89	0.86	0.87	0.87	0.87
D at 344mμ	0.083	0.137	0.180	0.315	0.472	
$R_2 = D_{344}/D_{344}$	1.0	1.0	1.0	1.0	1.0	1.0
D at 360 mμ	0.062	0.096	0.122	0.229	0.340	
$R_3 = D_{360}/D_{344}$	0.74	0.70	0.68	0.73	0.72	0.71
D at 370 mμ	0.034	0.052	0.069	0.125	0.185	
$R_4 = D_{370}/D_{344}$	0.41	0.38	0.38	0.39	0.39	0.39

* D values are the observed values after substraction of the corresponding values at $t=0$. The molar extinction coefficient ε of the pure *trans*-isomer is 24900, 28700, 20400 and 11100 at 332, 344, 360 and 370 mμ, respectively.

We conclude therefore, that carbonyl-methylene condensations, at least those of aromatic aldehydes with cyanoacetic esters, are subject to steric control resulting in the formation of the *trans* isomers.

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ACID–CATALYZED ALKYL–NITROGEN SOLVOLYSIS (A_{AL}) OF N-TRIPHENYLMETHYLACETAMIDE

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ABSTRACT

The kinetics of the cleavage: $CH_3CONHCPh_3 + H^+ \rightarrow CH_3CONH_2 + Ph_3C^+$ was measured spectrophotometrically in anhydrous mixtures of acetic-sulfuric acid. The reaction was found to be first order in the amide. No linear correlation was found between $\log k_1$ and $\log C_{H_2SO_4}$ or H_0. A mechanism of the A_{AL} 1 type is proposed on the basis of the kinetic results.

A considerable amount of work was done on the acid-catalyzed acyl-nitrogen solvolysis (A_{AC}) of amides[1] to the corresponding carboxylic acids and amines. However, the other possible route for solvolysis of N-substituted amides, alkyl-nitrogen cleavage of the type

$$RCONHR' + HX \longrightarrow RCONH_2 + R'X$$

when X is a nucleophilic agent, was investigated only preparatively. Recently, Lacey[2] reported the acid-catalyzed hydrolysis of several amides of the type RCONHR' (where R' was a tertiary alkyl group) to the corresponding primary amide and products arising from the tertiary alkyl carbonium ions, *i.e.*, ethylenes, alcohols, rearrangement products or polymers. Earlier it was reported[3] that N-triphenylmethylacetamide gave triphenylmethanol on heating with 90% sulfuric acid. The reaction seems to be general in strongly acid media, when R' is a radical which tends to form a comparatively stable carbonium ion. No kinetic data on the reaction were reported, and the mechanism proposed was based only on synthetic data. According to Lacey[2b] the mechanism of the reaction is essentially unimolecular (A_{AL} 1):

1) $RCONHR' + H^+ \rightleftharpoons RCONH_2R'^+$ fast equilibrium

2) $RCONH_2R'^+ \longrightarrow RCONH_2 + R'^+$ slow, rate determining

3) $R'^+ \longrightarrow$ products

In order to prove the mechanism we measured the kinetics of the reaction. For our measurements we chose an amide which forms on cleavage a relatively stable carbonium ion, *i.e.*, N-triphenylmethylacetamide. It is easy to follow kinetically the formation of the triphenylmethyl carbonium ion, owing to its characteristic absorption spectrum in the visible region and its stability.

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RESULTS

The cleavage of N-triphenylmethylacetamide was measured spectrophotometrically in anhydrous acetic acid solutions with varying amounts of anhydrous sulfuric acid. At each acidity, several measurements were carried out with different initial concentrations of the amide. The reaction was found to be first order in the amide. First order rate coefficients for the cleavage of various concentrations of the amide at constant acidity are given in Table I.

TABLE I

First order rate coefficient for the cleavage of $CH_3CONHCPh_3$, *at different initial concentrations* (C_0), *in acetic acid containing* 2% *(by volume) of* H_2SO_4 ($H_0 = -2.305$), *at* 40°

10^5C_0,	mole. 1^{-1}	7.27	14.53	18.87	21.81	29.07	36.37
10^4k_1,	sec^{-1}	3.01	3.04	3.01	2.99	2.99	3.01

The reaction was followed kinetically at a wide range of acidities at several temperatures between 30° and 46°C. The results are summarized in Table II; each value of k_1 in Table II is the average of 4–6 separate measurements at various concentrations of the amide.

No linear dependency was found between log k_1 either with H_o or with log $C_{H}{}^{+}$. The curves obtained are given in Figure 1.

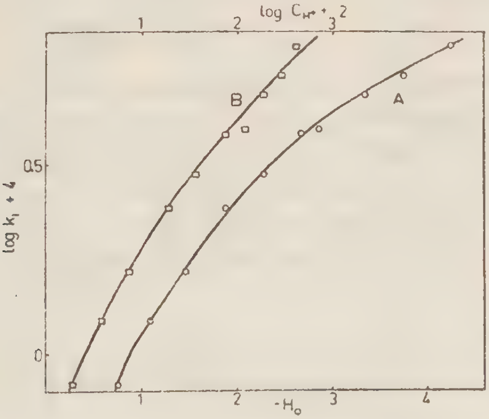

Figure 1: Dependency of log k_1 on Hammett's acidity function, H_0 (curve A), and the logarithm of the H_2SO_4 concentration, log C_{H^+} (curve B), for the cleavage of $CH_3CONHCPh_3$ at 40°.

Activation parameters were calculated from the rate coefficients at the temperatures mentioned. The values of log k_1 vs. 1/T lie on straight lines, as can be seen from Figure 2.

TABLE II

Cleavage of $CH_3CONHCPh_3$ at different acidities in acetic acid containing varying concentrations (C_{H^+}) of sulfuric acid, at different temperatures

C_{H^+}, mole.1^{-1}	0.01878	0.03758	0.0751	0.1878	0.3758	0.751	1.126	1.878	2.626	3.758
$-H_0$*	0.79	1.12	1.47	1.892	2.305	2.71	2.955	3.35	3.77	4.25
10^5k_1, sec^{-1} at 30°			4.45		8.84			14.2		
10^5k_1, sec^{-1} at 35°			8.71		16.9					
10^5k_1, sec^{-1} at 40°	8.16	12.5	16.6	24.5	30.1	38.4	39.5	48.0	55.0	65.5
10^5k_1, sec^{-1} at 46°					61.5			90.5		

* H_0 values were taken from the literature [14,16].

TABLE III

Energies of activation (E_{ac}) and entropies of activation ($\triangle S^*$) for the reaction at some acidities

$-H_0$	E_{ac} Kcal. Mole^{-1}	$\triangle S^*$ e.u.
1.47	24.65	+ 0.2
2.305	23.6	— 0.9
3.35	22.4	— 2.3

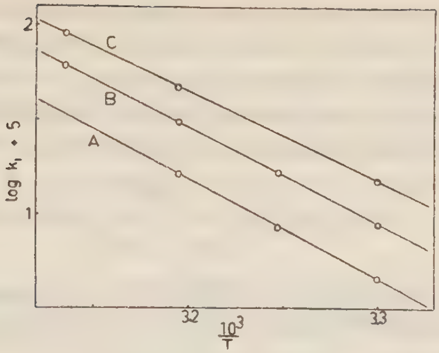

Figure 2: Dependency of log k_1 on 1/T. A, at H_0 = —1.47; B, at H_0 = —2.305; C, at H_0= —3.35.

The energies of activation for the different acidities were calculated from the slopes of the lines in Figure 2, and from them, using the absolute rate theory[4], the entropies of activation were calculated. The activation parameters are listed in Table III.

DISCUSSION

It was predicted[2b], on the basis of the proposed mechanism mentioned above, that the first order rate coefficients should be proportional to Hammett's acidity function values, h_o. According to Zucker and Hammett[5], for acid-catalyzed reactions, in which the rate determining step is unimolecular, log k_1 is linearly proportional to $-H_o$, *i,e.*,

$$H_0 + \log k_1 = \text{constant} \qquad \dots\dots\dots\dots \qquad (1)$$

This equation was obtained assuming that in the first protonation step of the reaction, *i.e.*, $B + H^+ \rightleftharpoons BH^+$, the ratio of the concentration of the conjugate acid BH^+ to the total concentration of the substrate is small. This assumption is correct only for very weak bases, especially at low acidities. When B is a stronger base, and the reaction is carried out at high acidities, the concentration of BH^+ cannot be ignored in comparison with the concentration of the substrate B. For such reactions, the general equation[6]

$$k_1 = \frac{k'}{K_{BH^+}} \cdot \frac{h_0}{1+h_0/K^{BH+}} = \frac{k'h_0}{K_{BH^+} + h_0} \quad \dots\dots \quad (2)$$

is valid, where k_1 is the empirical first order rate coefficient, k' is the rate coeffcient of the rate-determining step, and K_{BH^+} is the acidionization constant of BH^+.

Equation (2) reduces to (1) when $K_{BH^+} \gg h_o$, but when $h_o \gg K_{BH^+}$, *i.e.*, when B is a relatively strong base, and the reaction is followed at relatively strong acidities, then equation (2) reduces to

$$k_1 = k' \quad \dots\dots\dots\dots\dots \quad (3)$$

i.e., the rate coefficient approaches the limiting valuc, independently of acidity. It may be seen that in our reaction we have an intermediate case in which neither the concentration of the nonionized basic substrate, $[CH_3CONHCPh_3]$, nor of its conjugate acid $[CH_3CONH_2CPh_3^+]$, can be ignored.

Equation (2) is equivalent to equation (4) developed by Schubert and Latourette[7] for the deacetylation of sterically hindered aromatic ketones in strongly acid media:

$$\log k_1 - H_0 - \log \frac{[B]}{[B] + [BH^+]} = \text{constant} \quad \dots \quad (4)$$

In order to check the validity of this equation for our reaction, we had to know the values of [B] and of $[BH^+]$. These values can be calculated from the equation[8],

$$\log \frac{[B]}{[BH^+]} = H_0 - pK_{BH^+} \quad \dots\dots\dots\dots \quad (5)$$

The pK_{BH^+} of N-triphenylmethylacetamide cannot be measured at room temperature because of the speed of the decomposition of its conjugate acid media. We had therefore to use an approximate value of pK_{BH^+}. The pK_{BH^+} of acetanilide was reported[9] to be –0.91. As the triphenylmethyl group, compared with the phenyl group, is slightly more electron-attracting (as may be seen by comparing the acid dissociation constants of benzoic acid[10] $[pK_a = 4.20]$ and of triphenylacetic acid[11] $[pK_a = 3.96]$ at 25 °C), the N-triphenylmethylacetamide is a slightly weaker base than acetanilide, and its pK_{BH+} value was assumed to be –1.2. Using this value, we tested equation (4); the results are summarized in Table IV.

TABLE IV

Test of equation (4) *for the decomposition of* $CH_3CONHCPh_3$ *in acetic-sulfuric acid mixtures, at* 40°

$-H_0$	$-\log k_1$	$-\log \frac{[B]}{[B] + [BH^+]}$	$\log k_1 - H_0 - \log \frac{[B]}{[B] + [BH^+]}$
0.79	4.088	0.143	–4.74
1.12	3.904	0.263	–4.76
1.47	3.780	0.456	–4.79
1.892	3.610	0.772	–4.73
2.305	3.522	1.138	–4.69
2.71	3.420	1.524	–4.61
2.955	3.408	1.763	–4.60
3.35	3.318	2.153	–4.52
3.77	3.259	2.572	–4.46
4.25	3.187	3.050	–4.39

It can be seen that the sum in equation (4) is fairly constant for the lower and medium acidities. The small discrepancies in the higher acidities may be due both to the uncertainty of the pK_{BH^+} used for the calculations, and to changes, at high acidities, of the activity coefficients of the solutes, thus making equation (5) (which was used for the calculations) inapplicable. However, the data in Table IV fit fairly well the assumption of a unimolecular rate-determining step, although no linear relationship was found between log k_1 and H_0.

The small absolute values of the entropies of activation for the reaction at different acidities equally support a mechanism whose rate determining-step is unimolecular, *i.e.*, the transition state is not much more ordered than the initial state[12].

On the basis of our data we assume the mechanism of the reaction to be of an A_{AL} 1 type, *i.e.*, the mechanism proposed by Lacey[2b].

EXPERIMENTAL

Materials— Anhydrous acetic acid[13] and sulfuric acid[14] were prepared according to known procedures. Freshly prepared stocks of acid mixtures were used, in order to prevent "ageing"[15]. N-triphenylmethylacetamide was prepared by the reaction of triphenylmethanol with cyanoacetic acid or with acetamide[3].

Product Analysis— 0.75 g of $CH_3CONHCPh_3$ were dissolved in 50ml of 5% (by volume) H_2SO_4 solution in acetic acid, and the solution kept at room temperature for 24 hrs. Then it was poured into water and filtered. The white residue, 0.6 g (93%) was identified as triphenylmethanol by comparison with an authentic sample. The filtrate was neutralized by 20% KOH solution, evaporated on a steam bath, and the residue formed was extracted with boiling chloroform. After evaporation, the precipitate was crystallized from water and 0.11g (75%) of acetamide (identified by comparison with an authentic sample), was isolated.

Kinetic Measurements — The runs were followed in a Beckman DU spectrophotometer, in 1 cm fused silica cells placed in a thermostated cell compartment. The appearance of the absorption of the triphenylmethyl carbonium ion was measured at $\lambda = 406 m\mu$. The molar absorption coefficient, ε, was determined by assuming 100% dissociation of triphenylmethanol to trityl carbonium ions in concentrated sulfuric acid, and was found to be 36,900 (lit.[16] 36,400). The solutions were found to obey Beer's law. The rate coefficients were calculated from the slopes of the optical densities *vs.* time lines for the initial 10% of the reaction, by a method described[17].

Entropies of Activation, were calculated according to the absolute rate theory[4], $k_1 = kT/h \exp. (-\triangle H^*/RT) \exp. (\triangle S^*/R)$, when k_1 is the empirical first order rate coefficient, k is Boltzmann's constant, h is Planck's constant, $\triangle H^*$ is the enthalpy of activation, which equals E_{ac}–RT, R is the gas constant and T — the tempereture in °K.

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STUDIES ON THE ENZYMATIC HYDROLYSIS OF POLY-α-AMINO ACIDS

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ABSTRACT

Pepsin and rennin digest poly-L-glutamic acid in the *p*H range 2.0 to 5.0. Triglutamic acid is the major product of exhaustive hydrolysis. Diglutamic acid and glutamic acid are formed in trace amount. High oligopeptides (Glu_4 to Glu_9) are formed in the initial stages of the enzymatic hydrolysis. Synthetic glutamyl oligopeptides (Glu_3 to Glu_8), as well as their corresponding *N*-benzyloxycarbonyl derivatives are also hydrolyzed by pepsin, yielding mainly triglutamic acid, at a rate increasing with chain length.

Both poly-L-glutamic acid, in the *p*H range of 4 to 8, and poly-L-lysine, in the *p*H range of 7 to 12, are hydrolyzed by ficin. On exhaustive hydrolysis, poly-L-glutamic acid yielded mainly diglutamic acid and triglutamic acid, higher oligopeptides being formed at early stages of enzymatic digestion. Synthetic glutamyl oligopeptides are also hydrolyzed by ficin. In the hydrolysis of poly-L-lysine by ficin, Lys_3, Lys_4 and Lys_5 are formed. Papain hydrolyses poly-L-lysine at *p*H 9.2 in a manner similar to that of ficin.– High lysine oligopeptides were detected in tryptic hydrolysates of poly-L-lysine.

The results obtained suggest that the five endopeptidases studied act both on the helical and random coil forms of the poly-α-amino acids serving as substrates. An "intermediate" mechanism of hydrolysis was postulated.

INTRODUCTION

The recent studies on the enzymatic digestion of proteins with known amino acid sequence[1–5] have shown that the specificity requirements of proteolytic enzymes differ in many cases from those formulated by Bergmann, Fruton and their co-workers[6] on the basis of their studies with synthetic low molecular substrates.Trypsin, for example, exhibits almost the same specificity towards low or high molecular weight substrates, *i.e.* causes cleavage of lysyl and arginyl peptide bonds. Pepsin, however, exhibits a markedly broader specificity with proteins than with synthetic, low molecular weight substrates. From studies with the latter it was concluded that the bonds which are particularly susceptible to peptic digestion are those between two aromatic amino acids or between the α-carbonyl of a dicarboxylic amino acid and the α-amino group of an aromatic amino acid[6]. In proteins, however, pepsin was found also to hydrolyze peptide bonds between some of the aliphatic amino acids, as well as between other amino acids[1–5,7,8]. Of particular interest in this connection are the findings that bonds between the same amino acids (*e.g.* Ala-Ala

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and Val-Ala in oxidized ribonuclease) can be hydrolyzed by pepsin in one part of the protein molecule, but not in another part of the molecule[5,7].

Proteolytic enzymes have been shown to hydrolyse readily only denatured proteins, while native proteins are as a rule resistant to enzymatic digestion[9,10]. This finding indicate that the secondary and tertiary structure of the protein molecule may profoundly influence its susceptibility to enzymatic attack. Studies with low molecular weight substrates can, of course, shed no light on the effect of the conformation of the polypeptide chain on enzymatic susceptibility.

Synthetic, water-soluble, high molecular weight polypeptides have been shown recently to possess a helical conformation in solution[11]. Since a great variety of such polymers with known primary structure are now available[12] they can be used as suitable model compounds for the investigation of the mode of action of proteolytic enzymes on proteins. Some of the synthetic polyamino acids may serve better than low molecular weight peptides as substrates for determination of proteolytic activity of proteases. Synthetic polypeptides may also be of use in the search for new proteolytic enzymes[14].

Since the first observation that poly-L-lysine is readily digested by trypsin[14], first made in Prof. M. Frankel's laboratory in 1948, a considerable number of other amino acid polymers and copolymers have been tested for their susceptibility to enzymatic hydrolysis[15]. The results obtained with amino acid homopolymers are summarized in the Discussion (Table II). Proline iminopeptidase[13], a new enzyme discovered in our laboratory with the aid of poly-L-proline, is included among the enzymes listed in Table II.

Until recently only three cases, namely that of digestion of poly-L-lysine by trypsin[16], hydrolysis of poly-L-glumatic acid by papain[17], and hydrolysis of poly-L-proline by proline iminopeptidase[14], have been investigated in some detail. In this publication we present data on the action of pepsin, rennin, ficin and papain on a variety of amino acid polymers. Some new data on the mechanism of the tryptic hydrolysis of poly-L-lysine have also been included.

EXPERIMENTAL

Materials

Commercial preparations of crystalline pepsin (Mann Research Laboratories, N.Y.), papain (Worthington Biochemical Corporation, N.J.), and trypsin (Worthington Biochemical Corporation, N.J.) were used. Crystalline rennin in 5M NaCl was obtained from Christian Hansen Laboratories, Copenhagen. Ficin was prepared from dried fig latex powder (Nutritional Biochemical Corp.) by the procedure of Bernhard and Gutfreund[18].

The poly-α-amino acids used were prepared by the polymerization of the corresponding *N*-carboxy-α-amino acid anhydrides[12]. Protecting benzyl and benzyloxycarbonyl groups were removed with anhydrous hydrogen bromide in glacial acetic acid[19]. The copolymers used were of the random type. The individual macromolecules of these preparations may thus differ not only in chain-length but also in composition and sequence of constituent amino acids.

The L-lysine oligopeptides, Lys_n where $n=2$, 3, 4 or 5, were prepared according to the literature[20]

The α,L-glutamic acid oligopeptides, Glu_n where $n=2$, 3, 4, 5, 6, 7 or 8, were prepared by coupling *N*-benzyloxycarbonyl-γ-ethyl L-glutamate with the appropriate oligo-L-glutamyl ethyl ester, using the mixed anhydride method; removal of the ester groups with sodium hydroxide, and decarbobenzoxylation by means of anhydrous hydrogen bromide. Details of synthesis will be published elsewhere[21]. It was found that the glutamic acid oligopeptides can be separated on paper either by chromatography using *n*-propanol-ammonia as solvent, or by high voltage electrophoresis at *p*H 3.5 (see Methods). The mobilities of the different oligopeptides on paper chromatograms and electrophorograms are given in Table I.

TABLE I

Paper chromatographic and electrophoretic mobilities of glutamic acid oligopeptides

Compound	Paper chromatography[a] R_{Glu}	Paper electrophoresis[b] Distance towards anode (+) or cathode (—)
Glutamic acid	1.0	+ 1 cm
Glu_2	0.65	— 11 cm
Glu_3	0.50	— 8 cm
Glu_4	0.35	— 4.5 cm
Glu_5	0.22	— 3 cm
Glu_6	0.18	— 1.2 cm
Glu_7	0.14	— 0.5 cm
Glu_8	0.09	

[a] Solvent: *n*-propanol-water-concentrated aqueous NH_3, 100:50:1, v/v. Paper: Whatman No. 1. Room temperature.

[b] Pyridine-acetate buffer, *p*H 3.5, for 3 hours at 50 volt/cm; Whatman No. 1 paper.

All of the glutamic acid oligopeptides gave a positive ninhydrin reaction with a molar color yield approximately equal to that of glutamic acid[21].

Methods

Enzymatic Reaction Mixtures. The enzymatic studies were as a rule carried out in aqueous solution at 35° or 37°. A typical reaction mixture contained 5 to 10mg polypepdite and 25 to 500 μg. enzyme per ml. No buffers were used when the digestion was followed by the *p*H-stat method. In some of the other cases, 0.1M acetate, 0.1M citrate, 0.1M phosphate or 0.1M veronal buffers[22] were employed. In the acid *p*H range in which some of the glutamyl and aspartyl polymers are water-insoluble, dissolution was effected in the minimal amount of 0.1N NaOH, and the required pH was obtained by adding 0.1N hydrochloric, acetic or citric acids. A fine suspension of the substrate was thus obtained and the enzymatic digestion carried out in a heterogeneous system. In the experiments with ficin and papain, the enzymes were activated by 2,3-dimercaptopropanol (BAL) at a concentration of 10^{-3} M.

Analytical Techniques. The digestion was followed by measuring (a) increase in color produced with ninhydrin[23], (b) increase in Van Slyke amino-N[24], and (c) uptake of acid or base in the pH-stat[25]. The latter technique was applied only at pH 2.0 to 2.5 (titration with hydrochloric acid) or at pH 7.5 to 9.0 (titration with sodium hydroxide). Fair agreement was found in the results obtained by the three methods mentioned above.

The products of the enzymatic digestion were separated by high voltage electrophoresis or by paper chromatography and revealed with ninhydrin. Glutamic acid oligopeptides, formed in the enzymatic hydrolysis of the glutamic acid polypeptides, were separated electrophoretically within three hours on Whatman No. 1 paper, at a potential gradient of 50 volts/cm, using pyridine-acetate buffer, pH 3.5 (10 ml acetic acid, 1ml pyridine and 189ml water). For paper chromatography of the glutamyl oligopeptides, *n*-propanol-water-concentrated aqueous NH_3 (100:50:1 v/v) was used as solvent and Whatman No. 1 as the supporting medium. Descending chromatograms were run at room temperature for 48 to 72 hours. For the chromatographic separation of the lysine oligopeptides formed in poly-L-lysine digests, the solvent system acetic acid-*n*-butanol-pyridine-water (6:30:20:24 v/v)[16] was used.

RESULTS

Hydrolysis of polypeptides containing L-*glutamyl residues by pepsin*

The action of pepsin at a concentration of 0.3mg per ml, at pH 2.3, to 4.7, on a variety of poly-α-amino acids has been investigated[26]. Among the homopolymers tested poly-L-glutamic acid was the only one which hydrolysed under the experimental conditions used. Approximately 16 per cent of the peptide links of a poly-L-glutamic acid preparation, with an average degree of polymerization $n = 125$, were hydrolyzed within 30 minutes at pH 2.3. Only a small increase in the extent of hydrolysis occurred on additional incubation for 24 hours. From the uptake of acid at pH 2.3, an initial rate of hydrolysis of 3.0μmoles peptide bond per minute per mg of pepsin per ml was calculated, assuming a negligible extent of ionization of the newly formed carboxyl groups. A different preparation of poly-L-glutamic acid with an average degree of polymerizarion $n = 55$ was digested by pepsin at a similar rate. The initial rates of hydrolysis were proportional to concentration of enzyme within the range of 0.1 to 1.0mg pepsin per ml. The rate of enzymatic hydrolysis of poly-L-glutamic acid ($n = 125$), as determined by the ninhydrin method, decreased approximately 100-fold on increasing the pH from 2.3 to 4.5.

Oligopeptides containing 4 to 9 glutamyl residues could be detected, by paper chromotagraphy and by paper electrophoresis, in peptic digests of poly-L-glutamic acid after short incubation periods (10 to 30min.). Higher oligopeptides, although not identified, seemed also to be present. Triglutamic acid was found in trace amounts. Prolonged incubation periods (24 hours) resulted in complete disappearance of the high oligopeptides ($n = 6$ to 9) and formation of triglutamic acid in large amounts. The 24 hour digests contained, in addition to triglutamic acid, small amounts of the corresponding tetra- and pentaglutamylpeptides, and traces of glutamic acid and diglutamic acid. The chromatographic patterns of the digest at *p*H 2.3 were qualitatively similar to those of digests at *p*H 4.7.

The action of pepsin on synthetic glutamyl oligopeptides at *p*H 2.3 and 4.7 was next investigated. At the enzyme concentration employed (0.3 mg per ml), di-, tri-, and tetra-L-glutamic acid were practically resistant to hydrolysis. Penta-, hexa-, hepta-, and octaglutamic acid, however, were hydrolysed, at rates increasing with chain-length. Triglutamic acid was the major product formed as a result of the enzymatic hydrolysis of these oligopeptides. It is noteworthy that when the concentration of pepsin was increased to 5.0 mg per ml the tetrapeptide was also hydrolysed, although the di- and triglutamic acid remained intact. At this high enzyme concentration tetra-L-glutamic acid yielded glutamic acid, triglutamic acid and small amounts of diglutamic acid. The amount of free glutamic acid was considerably smaller than that of the tripeptide.

In order to determine whether the resistance to peptic digestion of the short glutamyl oligopeptides is due to an inhibitory effect of the α-NH_2 terminal groups, the action of pepsin on *N*-benzyloxycarbonyl-L-glutamyl oligopeptides[21] (Z. Glu_n, $n = 2$ to 7) was tested. It was found that the blocking of the terminal amino groups did not markedly effect the susceptibility of the peptides to hydrolysis by pepsin both at *p*H 2.3 and *p*H 4.7. Z.Glu_2 and Z.Glu_4 were resistant to pepsin both at low (0.3 mg per ml) and at high (5.0 mg per ml) enzyme concentrations. Z.Glu_4 was only partially hydrolyzed at high enzyme concentration, to yield triglutamic acid and presumably Z.Glu (not identified). Z.Glu_5, Z.Glu_6, and Z.Glu_7 were readily hydrolyzed, similarly to the corresponding unblocked oligopeptides, to yield triglutamic acid as the main product.

Copolymers of L-glutamic acid with D-Glutamic acid, L-aspartic acid, L-lysine, L-alanine, L-tyrosine or L-phenylalanine, were also tested for susceptibility to peptic digestion. Copolymers with relatively high L-glutamic acid content, such as L-Glu: D-Glu (molar residue ratio 11:1), L-Glu:L-Asp (9:1) or (5:1), and L-Glu:L-Tyr (9:1), were hydrolyzed at a rate and to an extent similar to those of poly-L-glutamic acid[26]. Copolymers with relatively low contents of L-glutamic acid were hydrolyzed at slower rates and to lesser extents. Tyrosine and phenylalanine residues, known to provide peptide links which are readily attacked by pepsin, decrease considerably the susceptibility of the corresponding L-glutamyl copolymers to peptic digestion.

*Hydrolysis of poly-*L*-glutamic acid by rennin*

Rennin closely resembles pepsin in its activity on poly-L-glutamic acid and L-glutamyl amino acid copolymers[21]. Chromatographic analysis of 24 hour rennin digests of poly-L-glutamic acid at *p*H 2.3 or *p*H 4.2, revealed, similarly as with pepsin, the presence of tri-, tetra- and pentaglutamic acids (see Figure 1). Triglutamic acid was the main product formed. Hydrolysis by rennin of poly-L-glutamic acid for short incubation periods resulted in the appearance of a series of higher glutamyl oligopeptides (Glu_3 to Glu_9).

The action of rennin at *p*H 4.2 on the various synthetic glutamyl oligopeptides has also been investigated. At an enzyme concentration of 1.7 mg/ml no digestion of diglutamic acid (10 mg/ml) could be detected within 48 hours. Under similar conditions, triglutamic acid gave diglutamic acid in small yield. Tetraglutamic acid was hydrolyzed considerably faster, yielding mainly triglutamic acid. The higher glutamyl oligopeptides (Glu_5 to Glu_8) were even more susceptible to rennin than the tetrapeptide and yielded mainly triglutamic acid after extended incubation. The octapeptide (Glu_8), 10 mg/ml, for example, disappeared from a reaction mixture (*p*H 4.2) containing 0.2 mg rennin per ml, already within 2 hours. In the enzymatic digests of Glu_3 to Glu_8, no glutamic acid could be detected, while diglutamic acid appeared only in trace amounts.

Carbobenzoxylated glutamyl oligopeptides ($Z.Glu_2$ to $Z.Glu_7$) behaved towards rennin similarly to corresponding unblocked peptides. The terminal α-amino group does not seem therefore to affect the susceptibility towards rennin of neighbouring peptide bonds.

A quantitative comparison of the activity of rennin with that of pepsin indicates that the former enzyme is considerably more active towards glutamyl polypeptides than the latter. Furthermore, the two enzymes seem to differ in their *p*H activity curves; maximal activity was attained with rennin at a *p*H value (*p*H of about 3.8) higher than that which characterizes the action of pepsin.

*Hydrolysis of poly-*L*-glutamic acid and of poly-*L*-lysine by ficin*[27]

Poly-L-glutamic acid ($n = 125$) is hydrolyzed by ficin (0.03 mg/ml) at the *p*H range of 4.0 to 8.0. Oligopeptides containing two to nine glutamic acid residues could be detected on paper chromatograms and electrophorograms after an incubation period of 30 minutes. Further incubation resulted in the conversion of the higher oligopeptides to di-, tri-, tetra- and pentaglutamic acids. Diglutamic acid and triglutamic acid were the major products after enzymatic digestion for 24 hours.

The action of ficin on glutamyl oligopeptides at *p*H 7.0 was also studied. Diglutamic acid was unchanged even after prolonged incubation periods (24 to 48 hours) with the enzyme at high concentration (6 mg/ml). Triglutamic acid could be hydrolyzed only to a small extent even by incubation for 24 hours with 3 mg/ml of ficin. Diglutamic acid, and trace amounts of glutamic acid were found in the digest. Tetra-,

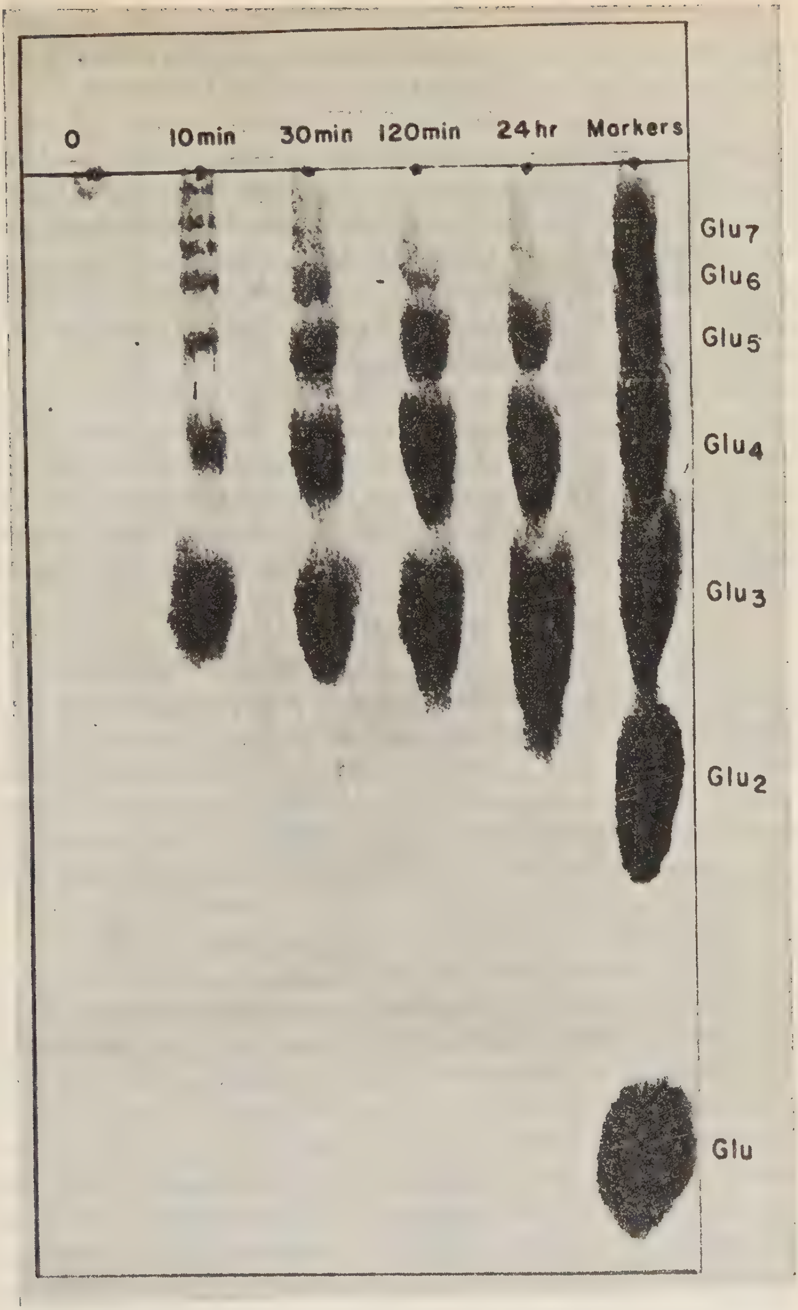

Figure 1

The action of rennin on poly-L-glutamic acid

The reaction mixture contained 5 mg of the polymer and 0.1 mg of enzyme in one ml of 0.1 M acetate buffer, *p*H 4.2 and was incubated at 37°. At the times given, 0.2 ml aliquots were withdrawn, mixed with 0.1 ml 0.1N NH_4OH to stop the enzymatic reaction, and analysed chromatographically. Twenty-five microliters of a diluted aliquot were used for each analysis. *n*-Propanol-water-concentrated-ammonia (100:50:1 v/v) was used as the developing solvent, the descending chromatogram run for 70 hours and the spots revealed with ninhydrin.

penta- and hexaglutamic acids were readily digested, at a rate increasing with chain length. The hexapeptide was hydrolyzed at a rate similar to that of poly-L-glutamic acid. All the oligopeptides studied yielded di- and triglutamic acid as the main products of hydrolysis. Glutamic acid, whenever present, appeared in trace amounts.

Determination of the *p*H dependence of the rate of hydrolysis of poly-L-glutamic acid by ficin, using the Van-Slyke amino-*N* method, showed maximal activity at about *p*H 6.0. The activity on the high molecular weight substrate decreases at *p*H 7.0 to about half of its rate at *p*H 6.0. Most of the enzymatic activity towards poly-L-glutamic acid is retained at *p*H 4.0 to 6.0. At *p*H 4.0 and 5.0 an activity equal to about 70% of that at *p*H 6.0 was recorded.

Poly-L-lysine was also digested by ficin[27]. Hydrolysis was found in the *p*H range of 7.0 to 12.0. The digest contained in addition to the unreacted polymer, traces of lysine and dilysine, and lysyl oligopeptides, Lys_n, with $n = 3$ to 10. Relatively high concentrations of ficin were required to attain an appreciable extent of hydrolysis. Thus even at a ficin concentration of 0.5 mg/ml at *p*H 9.0, degradation products could be detected in considerable amounts only after 24 hours, but not after 3 hours, of incubation.

*Hydrolysis of poly-*L*-lysine by papain*

Papain resembles ficin in many of its enzymatic properties[28]. Baker and Stahmann[29] were the first to show that poly-L-glutamic acid is hydrolyzed by papain. The effect of the conformation of poly-L-glutamic acid on the rate of digestion has been recently investigated[17]. We have found that papain can hydrolyse poly-L-lysine, similarly to ficin[27]. The chromatographic patterns obtained were similar in both cases. Preliminary experiments indicate that the rate of digestion of poly-L-lysine by papain at *p*H 9.2 is greater than that by ficin but considerably smaller than that by trypsin.

*Intermediates in the tryptic hydrolysis of poly-*L*-lysine*

In the study of the action of trypsin on poly-L-lysine, Waley and Watson[16] did not detect high lysine oligopeptides in any of their enzymatic hydrolysates. A selective cleavage of the peptide bonds near the end of the polymer chain has therefore been assumed. In view of our findings that high oligopeptides are formed in the initial stages of the digestion of poly-L-lysine by ficin and papain, it seemed of interest to reinvestigate the action of trypsin on poly-L-lysine.

On incubating poly-L-lysine (22 mg/ml) at *p*H 7 6 with trypsin (0.01 mg/ml) at an enzyme concentration lower than that used by Waley and Watson (0.03 mg/ml)[16] digests were obtained in which oligopeptides containing 2 to 9 lysine residues could be detected in the initial stages of the reaction (Figure 2)[27]. The high oligopeptides and the original polymer disappeared from the reaction mixture when the incubation period was prolonged. The final products of hydrolysis obtained after 24 hours were di-, tri-, and tetralysine in accord with the findings of Waley and Watson[16]. The high lysine oligopeptides could be detected in the digest even after 24 hours when very low trypsin concentrations (0.001 mg/ml) were used.

DISCUSSION

The results presented here show that various amino acid polymers and copolymers may serve as suitable high molecular substrates for pepsin, rennin, ficin and papain. The new data obatined, together with the previous findings on the enzymatic digestion of synthetic poly-α-amino acids are summarized in Table II. The acid proteases pepsin and rennin displayed similar specificity towards the high molecular weight synthetic substrates tested. Furthermore, both enzymes hydrolyzed poly-L-glutamic acid, as well as the synthetic glutamyl oligopeptides, to similar products. Great similarity was also observed between the two thiol-proteases, ficin and papain. Both enzymes digest acidic poly-L-glutamic acid as well as the basic poly-L-lysine in appropriate *p*H-ranges. Similar products were obtained when either of the above polymers was incubated with ficin and papain, respectively (see Table II).

The finding that poly-L-glutamic acid is digested by pepsin or rennin, shows that peptide bonds between glutamyl residues are highly susceptible to attack by either enzyme. The enzymatic cleavage of Glu-Glu bonds in proteins and native polypeptides has, however, not been reported[7,8]. The observation that copolymers of glutamic acid with aspartic acid, phenylalanine or tyrosine, are hydrolyzed by pepsin

TABLE II

*Hydrolysis of synthetic poly-α-amino acids by proteolytic enzymes**

Polyamino acid	Enzyme	*p*H	digested(+) or not digested(—)	Major products of hydrolysis	References
Poly-L-lysine	Carboxypeptidase	8.0–8.2	—		30
	Carboxypeptidase B	8.0–8.2	+	Lys	30
	Fibrinolysin (bovine)	7.4	+	Lys_2, Lys_3 Lys_4	31
	Ficin	7.0–12.0	+	Lys_3, Lys_4, Lys_5	27
	Papain	9.2	+	Lys_3, Lys_4, Lys_5	27
	Pepsin		—		14
	Thrombin	7.8	—		31
	Trypsin	7.6	+	Lys_2, Lys_3	16
Poly-D-lysine	Trypsin	8.0	—		32
Poly-L-ornithine	Trypsin		—		15
Poly-L-glutamic acid	Carboxypeptidase	5.0	+	Glu	29
	Chymotrypsin		—		29
	Ficin	4.0–7.5	+	Glu_2, Glu_3	27
	Papain	4.0–8.0	+	Glu_2, Glu_3	17, 29
	Pepsin	2.0–5.0	+	Glu_3, Glu_4	26
	Rennin	2.0–5.0	+	Glu_3, Glu_4	21
Poly-L-aspartic acid	Pepsin	2.3	—		26
Poly-L-proline	Proline iminopeptidase	7.8–9.5	+	Pro	13

* In addition to the purified enzymes listed, the following crude enzymatic preparations have been shown to act on synthetic poly-α-amino acids: Takadiastase hydrolyzed poly-L-lysine at *p*H 5 and poly-L-histidine at *p*H 5.3 to the corresponding free amino acids[31], and a pancreatic extract hydrolyzed poly-D-lysine at *p*H 6.0[32].

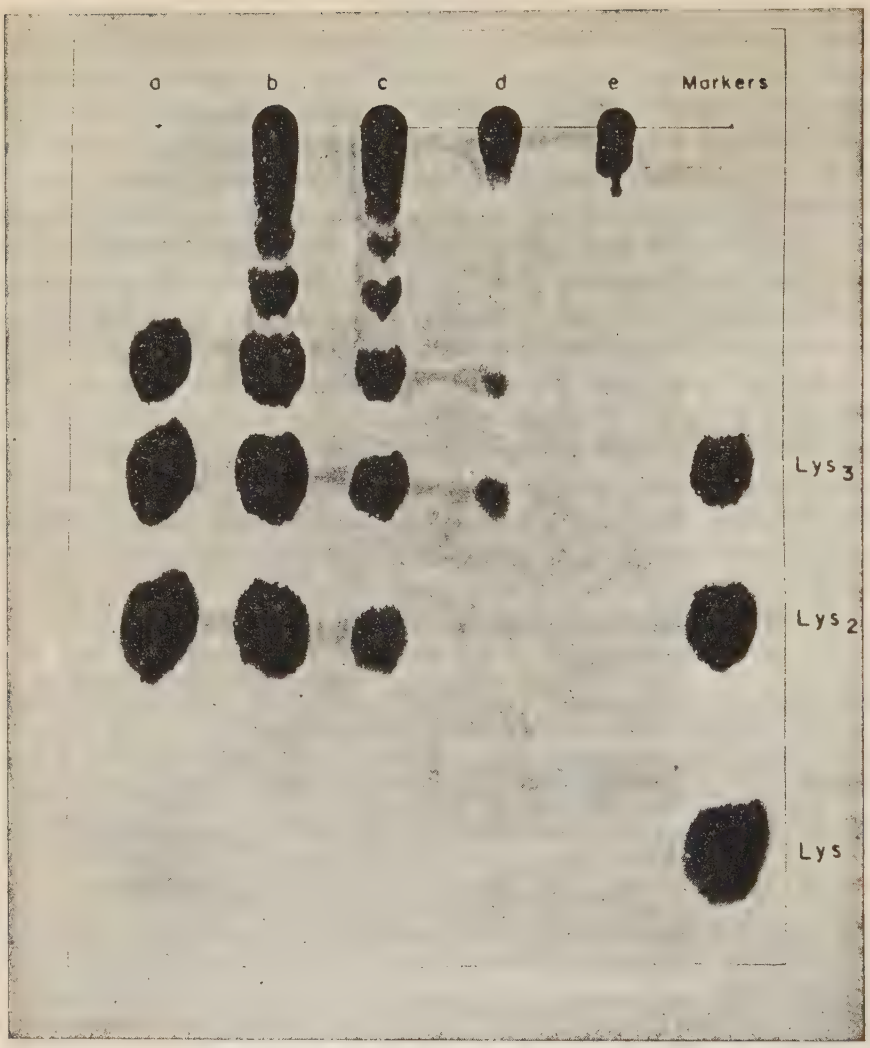

Figure 2

*The action of trypsin on poly-*L*-lysine*

Each reaction mixture contained 22 mg/ml of poly-L-lysine hydrobromide ($n=300$) in a solution 0.04 M in phosphate buffer, pH 7.6 and 0.083 M in KCl. Enzyme concentrations: (a) 0.043 mg/ml; (b) 0.011 mg/ml; (c) 0.004 mg/ml; (d) 0.001 mg/ml; (e) no enzyme. The reaction mixtures were incubated for 1 hr at 37°, and twenty microliters of each were then spotted on Whatman No. 1 paper. A descending chromatogram was run for 48 hours at rooom temperature using acetic acid-n butanol-pyridine-water (6:30:24:20 v/v) as solvent[16].

less readily than poly-L-glutamic is somewhat surprising since peptide links involving the above amino acids in proteins are attacked by pepsin[7,8]. The relative resistance of the glutamyl-amino acid copolymers to pepsin re-emphasizes the important role of amino acid composition and sequence in determining the susceptibility of a given peptide link to particular enzymatic hydrolysis. It is pertinent to note that poly-L-aspartic acid cannot be digested by pepsin, while the Asp-Asp bond in α-ACTH is hydrolyzed by the same enzyme[2].

The glutamyl oligopeptides Glu_2 to Glu_8, synthesized for this purpose, proved most useful in the clarification of the course of hydrolysis of poly-L-glutamic acid by pepsin and rennin. Di- and triglutamic acid are practically resistant to these enzymes. The higher oligopeptides are hydrolyzed at rates increasing with chain length; the rate of enzymatic hydrolysis of the hepta- and octaglutamyl peptides approaching that of poly-L-glutamic acid. The latter oligopeptides as well as polyglutamic acid hydrolyse at rates very much greater than that of the usual low molecular weight synthetic substrates of pepsin, *e.g.* acetyl-L-phenylalanyl-L-tyrosine[34] or *N*-benzyloxycarbonyl-L-glutamyl-L-tyrosine[35] in the case of pepsin, or *N*-benzyloxycarbonyl-L-glutamyl-L-tyrosine[21,36] in the case of rennin. Triglutamic acid was the major product of the enzymatic digestion of poly-L-glutamic acid and the larger glutamyl oligopeptides; glutamic acid and diglutamic acid appeared only in trace amount. This was the case also when the tetra- and pentapeptides were degraded enzymatically. It is thus plausible to assume that enzymatic hydrolysis of the glutamic acid peptides is accompanied by transpeptidation. Pepsin-catalyzed transpeptidations have indeed been demonstrated with synthetic low molecular weight substrates[37,38].

The formation of the high oligopeptides at the initial stages of the enzymatic hydrolysis of poly-L-glutamic acid, suggests that both pepsin and rennin act on the high molecular weight synthetic substrates by an "intermediate" mechanism[9]. Such a mechanism is characterized by a progressive degradation of the macromolecules with no specific susceptible bonds being either much more resistant or much more labile than the remaining susceptible bonds. Our experimental findings rule out for pepsin a "one by one" mechanism[9], in accord with the findings of Ginsburg and Schachman[39] for the peptic hydrolysis of ribonuclease.

Since the high molecular weight poly-L-glutamic acid is readily hydrolyzed by pepsin and by rennin at *p*H values (*p*H 2.0 to 4.0) at which the polymer exists in a helical conformation[40], it seems that both enzymes might act on polypeptides possessing a specific macromolecular sterochemical structure. A similar conclusion for pepsin was reached recently by Simons *et al.*[33] from studies of the action of the enzyme on poly-L-glutamic acid at *p*H 4.18. It is of interest, however, that glutamic acid oligopeptides ($n = 5$, 6, 7 and 8), which are devoid of a helical conformation[11], are also readily split by pepsin. It is thus possible that pepsin and rennin act on the helical poly-L-glutamic acid in a manner similar to that proposed for the action of proteolytic enzymes on native proteins[9,10,41], i.e. denaturation of the substrate promoted by the enzyme leading to the uncoiling of the polypeptide backbone, followed by an enzymatic hydrolysis of the "denatured" polypeptide.

The thiol proteases ficin and papain have been found to hydrolyze poly-L-glutamic acid (for the hydrolysis by papain see Miller[17]) and poly-L-lysine in the *p*H range of

4.0 to 8.0, and 7.0 to 12.0 respectively. Both enzymes can, therefore, cleave peptide bonds between two glutamyl residues as well as bonds between two lysyl residues. Studies with small substrates have shown that the best substrates for papain and ficin, such as α,*N*-benzoyl-L-lysine amide and α,*N*-benzoyl-L-arginine amide[28], are positively charged, while negatively charged substrates, such as benzyloxycarbonyl-L-isoglutamine or benzyloxycarbonyl-L-glutamyl-L-tyrosine[28], are generally hydrolyzed at much slower rates. In the case of the synthetic polymeric substrates, however, it was found that the highly negatively charged poly-L-glutamic acid is hydrolyzed at *p*H 7.0 by ficin more readily than the highly positively charged poly-L-lysine.

The oligopeptides formed on the digestion of poly-L-glutamic acid by ficin and papain resemble on the whole those obtained upon the hydrolysis of the same high molecular weight substrate by pepsin or rennin. It should be noted, however, that while triglutamic acid is the major product of the exhaustive enzymatic digestion of poly-L-glutamic acid by pepsin and rennin, diglutamic and triglutamic acid are the major products of the exhaustive digestion of the polymer by the thiol proteases. As a matter of fact, triglutamic acid, which is practically resistant to hydrolysis by pepsin and rennin, undergoes slow hydrolysis by ficin. A semi-quantitative analysis of the products formed upon the digestion of the glutamyl oligopeptides by ficin suggests that the hydrolysis is accompanied by a transpeptidation reaction similar to that observed in the case of pepsin and rennin.

The *p*H-dependence of the cleavage of poly-L-glutamic acid by ficin indicates that the polypeptide can be split enzymatically while in helical or random coil conformation. The data available do not permit, however, any conclusion as to the preferential cleavage at the helix–coil junctions, as suggested by Miller for the hydrolysis of poly-L-glutamic acid by papain[17].

Poly-L-lysine can also be split by ficin (*p*H 7.0 to 12.0) in its helical and random coil conformations[42]. The lysyl oligopeptides obtained were found similar to those identified by us in the tryptic digest of poly-L-lysine. In contrast to the case with trypsin, ficin acting on poly-L-lysine yielded only trace amounts of dilysine.

Study of the hydrolysis of synthetic poly-α-amino acids by five different endopeptidases has revealed that the suitable high molecular weight substrates are split at random to yield the corresponding high oligopeptides. The latter undergo further cleavage to give mainly trimers or dimers, but only small amounts of free amino acids. The experimental findings can be accounted for, in all cases, by an "intermediate" mechanism of hydrolysis[9]. Finally, it seems that the proteases investigated can attack synthetic polypeptides in their helical as well as random coil conformations, irrespective of the electric net charge of the substrate.

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